REVIEW ARTICLE

RECENT DEVELOPMENTS IN THE PHARMACY OF ANTIBIOTICS

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The unique therapeutic properties of penicillin, discovered by Florey and his Oxford collaborators in 1940, have led to some noteworthy advances in applied pharmacy. In particular they have set the industrial pharmacist diverse problems in making available a wide range of useful preparations for preventing and curing disease. Some idea of the extent to which this antibiotic is used to-day is illustrated by the following figures giving the annual production (all forms of penicillin) in the United Kingdom for the last five calendar years.[1]

	Mega-units millions
1947	4·881
1948	9·687
1949	18·502
1950	37·599
1951	63·166

Of the other valuable antibiotics since discovered, notably streptomycin, aureomycin, chloramphenicol and terramycin, none is so extensively used in this country as penicillin: moreover, none has offered so strong a challenge to the skill of the pharmacist. Thus, although streptomycin is of considerable importance in the treatment of diseases such as tuberculosis, it offers less scope for pharmaceutical ingenuity than penicillin because the range of its preparations is limited by its fewer clinical applications.

Again, although the so-called "wide spectrum" antibiotics, aureomycin, chloramphenicol and terramycin, are now recognised as valuable systemic chemotherapeutic agents, they are of less interest to the pharmacist in his role of formulator than is penicillin. The common route of administration for all three is by mouth, usually in capsules, and so the range of presentations is restricted. Although the newer antibiotics are said to be adequately absorbed from the alimentary tract, it is also true that, in contrast to penicillin, they possess physico-chemical properties rendering them less suitable for administration by injection.

Since the general discussion on penicillin at the British Pharmaceutical Conference, 1946, the extent of developments in the antibiotic field as a whole is truly remarkable. Indeed, expansion in knowledge has been so rapid that the subject is to-day vast and complicated. To attempt to do full justice to more than few of the many pharmaceutical developments

that have taken place in recent years is beyond the scope of this or any similar review. Accordingly, its main purpose is to describe such developments in broad terms except when advancements have been outstanding or are of special interest. It is convenient to deal with each antibiotic separately and under the following two sub-headings: (1) preparations for systemic therapy; (2) preparations for local therapy.

Penicillin

A development of considerable importance occurred at the beginning of 1947 when pure penicillin—as benzylpenicillin or crystalline penicillin G—first became available on a commercial scale in Britain. Apart from the obvious clinical advantages, this event also had repercussions on the pharmacy of penicillin. Increased thermostability and reduced hygroscopic properties constitute the main advantages of benzylpenicillin sodium and benzylpenicillin potassium over the impure freeze-dried salts formerly available. These qualities have eased the problems of the manufacturer, have made it possible to embark on universal distribution of the dry salts with an extended life of three years and have enabled the research pharmacist to formulate products that retain their potency in tropical as well as in temperate climates.

1. (a) *Preparations for systemic use—short acting.* By far the most important preparation of penicillin is the aqueous injection, in which form it exerts its maximum clinical effect. The sodium or potassium salts of benzylpenicillin (B.P. Addendum 1951) are customarily used for this purpose.

(i) *Stabilisation with buffers.* Although benzylpenicillin in the dry state is stable, being unchanged after several hours heating at 100° C., its keeping qualities in aqueous solution are poor.[2] Hydrolytic opening of the β-lactam ring occurs (hastened by rise in temperature) to yield irreversibly the inactive penicilloic acid, which reduces the *p*H and so catalyses further decomposition. This reaction can be slowed down by buffering at *p*H 6 to 7, the optimal *p*H for stability. Numerous workers have reported[3 to 13] their findings with citrate and phosphate buffers. The general consensus of opinion is that buffered solutions of benzylpenicillin retain their potency for significantly longer periods than unbuffered ones, whether stored at ambient temperatures or in the refrigerator; further, most workers consider citrates to be more effective than phosphates. A new development is reported in a contemporary communication[14] describing the stabilising effect of hexamine on simple and buffered solutions of sodium benzylpenicillin. In its presence longer periods of storage are possible than hitherto, and thus the work of the pharmacist is lessened in those hospitals where it is the practice to prepare solutions in bulk for issue to the wards. It is therefore unfortunate that the 1951 Addendum to the British Pharmacopœia, 1948, should permit the use of suitable buffering agents in making injection of penicillin and should at the same time reduce the period of storage in a *refrigerator* from 7 to 4 days. In view of the ample published evidence of the superior keeping qualities of buffered solutions, it would seem

desirable that the footnote to the existing monograph for injection of penicillin should be amended accordingly. Alternatively, it might be preferable to follow the example of the U.S.P. XIV, which includes buffered crystalline penicillin as a separate monograph.

(ii) *Stabilisation by other means.* Other investigators have attempted to improve the stability of penicillin solutions by incorporating substances known to sequester trace metals, of which copper, zinc and mercury in particular are known to attack the sulphur-containing ring in the penicillin molecule. Lester Smith[15] studied the effect of sodium hexametaphosphate; later Chain and Philpot[16] reported their findings with 2:3-dimercaptopropanol. More recently, Swallow[17] has described the stabilising action of sodium ethylenediamine tetra-acetate. A third and previously unreported approach to the stabilisation of penicillin solutions, by use of soil extracts, has been reported by Coulthard, Fawcet, Lewis and Sykes.[18]

(iii) *Bacteriostatics.* The ideal bacteriostatic for addition to penicillin solutions dispensed in multiple-dose containers has yet to be defined. The unusual sensitivity of the antibiotic to other substances, along with the need for more fundamental work on bacteriostatics in general, presents a number of difficulties. Of the many substances investigated up to date only phenylmercuric nitrate (0·001 per cent.) and phenol (0·5 per cent.) seem to justify serious consideration. In our laboratories the former has given good results and has the advantage of being as satisfactory in simple as in buffered solutions of penicillin. Phenol has been claimed[18] to exert a stabilising effect on solutions of penicillin G, but it lacks the utility of phenylmercuric nitrate, especially in citrate-buffered solutions, which deposit crystals of phenyl phenaceturate at room temperature.[19]

1 (b) *Preparations for systemic use—prolonged acting.* For several years commercially available forms of penicillin were restricted to the calcium, sodium, and potassium salts, all of which are highly soluble in water and tissue fluids and are therefore rapidly eliminated from the body. In consequence, many attempts have been made to decrease frequency of administration by delaying absorption or excretion of the antibiotic and so prolonging the action of injected doses. The familiar oil-beeswax suspension of penicillin introduced by Romansky and Rittman[20] is a typical example of modern formulation aimed at delaying the absorption of a water-soluble drug.

The popularity of this preparation was short-lived; unfavourable reactions at the sites of injection, associated with inherent difficulties in administration, quickly led to a search for something better. Accordingly, attention was focussed on the alternative and more physiological approach, prolonging the action of penicillin by its conversion into less soluble salts, esters or complexes. To-day many such compounds are known, but the only one that has so far come into widespread clinical use is procaine benzylpenicillin (B.P. Addendum, 1951). The approximate solubility of procaine penicillin G in oil and water is 7000 I.U./ml. As a result of this low solubility, the salt, when given intramuscularly as a

suspension in oil or water, produces a depot or slow release effect at the site of injection, thus prolonging the presence of the penicillin in the blood. To-day pharmaceutical manufacturers provide a multiplicity of procaine penicillin preparations for parenteral use; most of them contain 300,000 I.U. of penicillin combined with 120 mg. of procaine base in each 1-ml. dose. Some are available as ready-prepared suspensions in oil or water, others are presented as dry powders to which the aqueous diluent is added immediately before use. None, however, is yet included in the British Pharmacopœia. In discussing some of the more important pharmaceutical aspects of these preparations, it will be convenient to deal with them in the order of their origin and to follow the trend through to the most recent developments with the newer penicillin salts of the slow release type; some of them are reputed to have advantages over the procaine salt.

(i) *Simple and compound suspensions in oil.* Clinical studies on procaine penicillin in oil were first reported by Herrell, Nichols and Heilman[21] in 1947. There quickly followed commercial preparations in which was used refined sesame or arachis oil containing 300,000 I.U. in 1 ml. as free-flowing suspensions. With them, according to numerous clinical reports, therapeutic blood levels ($>$ 0·03 I.U./ml.) are obtained for 24 hours in most patients. Moreover such preparations need not be refrigerated, as at room temperature they retain their potencies for 12 to 18 months. Although it is a marked improvement on the earlier arachis oil-beeswax formulation, procaine penicillin in oil presents difficulties, because the procaine salt on standing separates from the oil, thus requiring vigorous agitation in order to re-establish the suspension. Several attempts have been made to facilitate easier re-suspension by incorporating small percentages of surface-active materials, such as ethylene glycol distearate or a polyoxyethylene derivative of a partial higher fatty acid ester of sorbitan (e.g., "tween 80").[22,23] These measures, however, were not completely successful; when Buckwalter and Dickison[24] added aluminium stearate to the mixture to provide a suspension of greatly improved physical stability and delayed absorption effect, the use of procaine penicillin in oil quickly declined in many parts of the world.

Procaine penicillin in oil with 2 per cent. aluminium stearate is of unusual interest, because problems of particle size and rheology are involved. The addition of the stearate not only stabilises the suspension but also ensures a therapeutic level in the blood over a much longer period than was possible with ordinary procaine penicillin in oil. According to a number of workers[24,25,26,27] this effect is most marked in man when the particle size of the penicillin is 50μ or less. Floyd[28] has since confirmed these findings in a mouse-protection test. Previous investigators[29,30] had shown the opposite effect and claimed that optimal prolongation resulted from the use of penicillin particles exceeding 50μ. The mechanism of extended slow-release by reduction of particle size is not known with certainty. Water-repellency of the stearate, controlled release of the penicillin from a conceivable latticework structure in the gel and

adsorbed films of "procaine stearate" have all been suggested as possible explanations: so far, there has been no confirmation of any. It is evident from an examination of commercial prototypes that they all conform to more or less the same standard for particle size—i.e., 90 to 100 per cent. under 5μ with substantially nothing in excess of 10μ. From the claims of the inventors,[31] who prefer procaine salts of penicillin G with a particle size of 0·1 to 10μ, it would seem that this degree of size-reduction is essential for the maximum prolongation effect. Supporting clinical investigations demonstrated that penicillin blood concentrations equal to or exceeding 0·03 I.U./ml. of serum were obtained in most patients after intramuscular injection of 1 ml. (300,000 I.U.) of the suspension. Tables are also included to illustrate the results in rabbits with the particle size reduced from 250μ to 1 to 2μ.

Thus some 30 per cent. w/v of solid penicillin with a high specific surface is incorporated in a viscous aluminium stearate gel. If the latter has been prepared to give the desired visco-elasticity, the resultant suspension may have the characteristics of a thixotropic gel system in which the dispersed-solid remains in substantially permanent suspension for a long time. A sharp rap and a few vigorous shakes are sufficient to reduce the preparation to a uniform, liquid state. In contradistinction, when similar suspensions are made with penicillin of low or relatively low specific surface the property of thixotropy may be reduced or lost altogether. Such preparations are more prone to sediment and impact and so to inconvenience users trying to re-establish the suspension. Especially is this liable to occur with mixtures of fine and coarse particles; the former, by virtue of their greater deformability and smaller mass are able to adhere to the latter, thus illustrating the experimentally established fact that a mixture of coarse and fine particles settles more quickly than do particles of uniform size. Although it would appear that a thixotropic product is preferable for maintaining the physical stability of suspensions, clinical experience has shown that equally satisfactory blood levels can be obtained from less viscous preparations that do not exhibit thixotropy.

Ultra-fine grinding of the procaine penicillin under sterile conditions with highly specialised machines operating on the fluid energy principle is necessary to give the size-reduction mentioned above. Again, the efficacy of the aluminium stearate gels depends on the care exercised over moisture content, viscosity and other properties, which are controlled by the heat treatment employed in their manufacture. Eastland[32] has correctly referred to the lack of precise technical information on the most suitable type of aluminium stearate. Reputable British manufacturers deny the existence of the hypothetical *mono*, *-di-* and *tri*-stearates of aluminium and claim that any desired ratio of aluminium to stearic acid can be obtained by varying the conditions of precipitation. The general belief is that aluminium stearate of commerce is an adsorption complex of stearic acid with alumina monohydrate. There is some support for this point of view in the literature,[33,34] and it would seem that the use of the description "mono-stearate" is not justified in any precise sense.

Although the duration of penicillin in the blood is prolonged after injections of procaine penicillin in oil with aluminium stearate, the peak concentration is low. In other words, the highest concentration of penicillin at any time after the injection is lower than that obtained with, say, 50,000 units of aqueous penicillin or 300,000 units of simple procaine penicillin in oil. This fact led to the idea of supplementing the sparingly soluble procaine salt with a dose of soluble penicillin G sodium or potassium, thereby combining the advantages of slow-release with a high initial concentration of penicillin in the blood. Several available commercial preparations of this type contain 300,000 I.U. of the procaine salt and 100,000 I.U. of soluble penicillin suspended together in each 1 ml. of arachis oil with aluminium stearate. On injection, this produces first a powerful action due to the soluble material, with a peak of 1·0 to 2·0 I.U./ml. of blood, and then a sustained effect due to the procaine salt, lasting 24 hours or more. Such preparations are said to be superior to procaine penicillin alone, providing a satisfactory clinical response in staphylococcal and other more resistant infections within the range normally considered susceptible to penicillin treatment. More recently, Welch and his associate workers[35] have shown that the blood concentrations of penicillin obtained by the addition of pectin-treated crystalline potassium penicillin gave higher maximum levels than those obtained by the addition of the potassium salt alone.

Aluminium stearate suspensions of procaine penicillin G retain their potency for at least 12 to 18 months at room temperature. Storage at elevated temperatures or in the refrigerator is contra-indicated, because exposure to extremes of heat or cold tends to accelerate ageing and breakdown of the gels. A disadvantage that applies equally to the plain and compound suspensions is that dry syringes and needles must be used for their administration. Except in the treatment of special diseases, such as syphilis and yaws, for which the aluminium stearate suspensions are still preferred by some sections of the medical profession, oily formulations have been largely superseded by the more convenient aqueous forms of procaine penicillin. The disadvantages of the former (including, *inter alia*, pain and indurations on injection, the possibility of oil embolisms, the need of exacting conditions for administration and the difficulty of cleaning the needle and syringe) fostered the search for improved presentations.

(ii) *Aqueous suspensions* (*for extemporaneous preparation*). The very early aqueous preparations of procaine penicillin G were dry products containing the antibiotic in association with a non-injurious surface-active agent. On adding sterile water or saline solution the surface-active agent wetted the crystals of procaine penicillin, thereby facilitating suspension and minimising the formation of clumps. Although the procaine salt was stable in the vehicle, it settled out with undesirable rapidity; when the suspension was administered from a syringe, the plunger tended to "freeze" in the barrel. This type of mixture was quickly succeeded by improved dry products containing a harmless hydrophylic colloid in addition to the surface-active agent. The combined effects of these

adjuvants facilitates rapid wetting of both the penicillin salt and the hydrophylic colloid, maintains the penicillin in discrete particulate suspension and minimises the danger of needle blockage from clumping on addition of the aqueous vehicle.

Non-ionic wetting agents are normally employed in formulating these preparations; the polyoxyethylene ethers of partial higher fatty acid esters of sorbitans (e.g., "tween 80") and suitable mixtures of the fatty acid esters of polyethylene glycol are typical examples. For the hydrophylic colloid many substances, such as gelatin, pectin, dextrin, alginates, tragacanth and the water-soluble salts of carboxymethylcellulose, have been suggested. Few, if any, however, satisfy all the criteria for the satisfactory manufacture of dry products for injection. For example, sodium carboxymethylcellulose has many desirable qualities and has been widely used as a suspending agent in the United States; some grades, however, have the disadvantage of being only slowly soluble in water, and this can sometimes give rise to difficulty when it is necessary to administer suspensions immediately after their preparation.

Present-day use of dry procaine penicillin for aqueous suspension is largely confined to those preparations containing a supplement of the soluble sodium or potassium salts of benzylpenicillin and a suitable buffering agent. Such mixtures normally contain concentrations of penicillin salts identical with those described in the previous section. Further, they have similar clinical applications but without the attendant disadvantage of oily vehicles. In the dry state, mixtures of procaine penicillin with buffered soluble penicillin have a shelf-life of 3 years. Sterile aqueous suspensions retain their potency for 7 days at room temperature or 14 days in a refrigerator. If the products are further stabilised[14] these periods can be doubled.

(iii) *Aqueous suspensions (ready prepared).* The low solubility of procaine penicillin G in water has been turned to advantage in formulating ready-prepared suspensions of the salt. Procaine penicillin is relatively stable in high concentrations in water, but it is not sufficiently so to withstand the exacting conditions demanded by commercial practice. Although by no means universally applied—since there are preferred methods of achieving the same end—one of the more interesting techniques for stabilising aqueous suspensions of procaine penicillin utilises the solubility-product principle,[36] that is, depression of solubility by common ion effect. The addition of a small excess of the cation procaine (as soluble salt) results in a significant reduction in the solubility of the procaine penicillin, thereby slowing down the rate of decomposition.

Stabilised aqueous suspensions of procaine penicillin contain buffering, suspending and wetting agents, of a similar type to those used in the dry presentations. That some commercial preparations are more stable than others is reflected in the different conditions laid down for their storage. Some are stated to be stable for 12 months at room temperature, whereas others are required to be kept below 15° C. (i.e., in a refrigerator). These differences are magnified at higher temperatures, as shown in Table I,

TABLE I

STABILITY OF AQUEOUS SUSPENSIONS OF PROCAINE PENICILLIN G STORED AT 40° C.

Origin of Product	Initial	Percentage Potency Remaining After										Final *p*H
		1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	10 weeks	11 weeks	
Europe	100	79	85	72	67	56	47	29	0	0	—	5·3
Great Britain ..	100	86	95	85	85	85	90	—	88	—	74	6·0
Great Britain ..	100	93	88	82	86	78	—	65	—	67	—	5·8
U.S.A...	100	91	78	79	86	83	—	70	—	42	—	5·4

TABLE II

HUMAN BLOOD SERUM LEVELS

N,N-Dibenzylethylenediamine Penicillin G—300,000 I.U. in Aqueous Suspension
1 ml. Dose

Patient	Normal	I.U./ml. in Blood Sample after Injection												
		1 hr.	6 hr.	12 hr.	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	144 hr.	168 hr.	192 hr.	216 hr.	240 hr.	264 hr.
A	0	0·037	0·037	0·042	0·058	0·051	0·037	0·034	0·020	0·015	0·015	0	0	0	0
B	0	0·062	0·042	0·061	0·072	0·048	0·041	0·035	0·035	0·023	0·020	0·015	0·015	0·015	0
D	0	0·018	0·015	0·016	0·021	0·015	0·018	0·017	0·020	0·018	0·016	0·015	0·015		
E	0	0·051	0·071	0·086	0·071	0·062	0·051	0·041	0·020	0·015	0·015	0·015	0·015	0·015	0·021
Average		0·042	0·041	0·051	0·055	0·044	0·037	0·032	0·024	0·018					

from Elias *et al.*[41]

which illustrates the comparative stability of four preparations (300,000 I.U./ml.) stored for 11 weeks at 40° C.

(iv) *Particle size.* The advent of antibiotic suspensions has focussed attention on the question of particle size. It is, however, a controversial subject and can receive but brief comment here. Aside from the specialised application of the oil/stearate suspension of procaine penicillin to the treatment of specific diseases, the clinical significance of particle size is debatable. On the one hand, there is the school of thought that insists on prolonged blood levels as necessary for effective treatment. Opposed to this is the view that maintenance of a continuous detectable level of penicillin is unnecessary and that the clinical efficacy of the preparation is the only test, not its ability to produce blood levels over long intervals of time.

Apart from therapeutic considerations, control of particle size is important to the pharmacist in arriving at a satisfactory formulation. In this respect, minimal sedimentation, ease of re-constitution and freedom from any tendency to block needles are essential criteria. Theoretically, it is desirable that all the particles should be of uniform size and shape. In practice, however, this is difficult to achieve and it is evident from an examination of various American and British preparations of procaine penicillin that standards vary considerably. For example, the range in some preparations is largely confined to 50 to 100μ. In others, the bulk of the material is under 25μ with only a few per cent. over that size and nothing in excess of 100μ. Again, other preparations show a fairly uniform spread over the whole range with little or nothing over 100μ. Generally speaking, it is desirable to limit the percentage of undersize and oversize material. Large crystals are obviously conducive to needle blockage. Too high a proportion of fine particles can also cause the same difficulty because of the tendency to form gelatinous agglomerates. Another disadvantage of fine particles is that they can complicate the formulation of dry parenteral products by giving rise to foaming problems.

(v) *Drain-clear containers for antibiotic suspensions.* A recent innovation of considerable novelty is the introduction of silicone-treated containers for aqueous liquid preparations. Although of fairly wide application, the invention[37] is of special interest in relation to antibiotic suspensions. It utilises the principle of rendering the internal surfaces of injection vials water-repellent by means of selected organopolysiloxanes, i.e., organo-silicon oxide polymers. The treatment is carried out with a solution of the appropriate silicone followed by evaporation of the solvent and baking of the silicone film under prescribed conditions. The main advantages of silicone treatment as applied to antibiotic suspensions are economy of the surpluses otherwise required to ensure adequate dosage, more accurate dosage and the improved æsthetic appeal of the drain-clear effect.

(vi) *Newer slow-release type salts of penicillin.* The utility of the sparingly soluble procaine benzylpenicillin lent considerable impetus to the search for similar slow-release salts. Until recently, however, little has been published since Monash[38] described the depot effect of various

penicillin salts other than those then in current use. Many new insoluble types—especially amine salts—are now receiving mention in the patent and general literature. Only two will be mentioned here, each of which, in its own way, depicts the modern trend in antibiotic depot therapy.

In the course of investigating salts of penicillin, Szabo *et al.*[39] observed that the penicillin salt of *N*,*N'*-dibenzylethylenediamine is nearly insoluble in water. Accordingly, this salt was selected for further examination in collaboration with associate workers.[40,41] Pharmacological and histological studies in animals after its injection in watery or oily media, accompanied by extensive feeding tests, showed that this salt (consisting of 2 moles of penicillin to 1 mole of base) compares very favourably with procaine penicillin (control). Subsequent studies in man were equally promising.

Dibenzylethylenediamine dipenicillin G has a theoretical potency of 1307 I.U./mg.; approximately 7 to 9 per cent. of water of crystallisation is present and results in practical potencies of 1180 to 1200 I.U./mg. The salt melts at about 110° C. and its solubility in water is approximately 200 I.U./ml.; that is, about 1/30 that of procaine penicillin. A striking advantage of the new salt is that it is tasteless and thus facilitates formulation of palatable suspensions for oral use (see later).

As shown by potency tests conducted over 12 months at various temperatures, dibenzylethylenediamine dipenicillin is at least as stable as procaine penicillin in all its current presentations. Although this is not specifically emphasised, the published evidence suggests that aqueous suspensions of the salt stored at elevated temperatures (37° C.) retain their potency much better than comparable preparations of procaine penicillin. This might be expected from the considerable differences in solubility.

Penicillin blood concentrations in human subjects after the intramuscular injection of dibenzylethylenediamine dipenicillin G in aqueous suspension have been reported[41] and Table II is abstracted from the published results. These show residual concentrations of penicillin over periods far in excess of any hitherto reported for slow-release antibiotic salts in aqueous suspension. Other results presented illustrate the cumulative effect produced by repeated intramuscular injection of suspensions of dibenzylethylenediamine dipenicillin (non-micronised) in water or in oil with aluminium stearate. Aside from oral therapy, and provided the initial work with this new salt is borne out in full-scale practice, it may well supplant procaine penicillin in some of its more specialised applications. For example, the oil/stearate injection of the latter could be replaced to considerable advantage by an aqueous suspension of one of the less soluble salts of penicillin.

Progress of a different but equally interesting nature has recently been reported by Longacre,[42] who described the clinical advantages of the penicillin salt of *N*-methyl-1:2-diphenyl-2-hydroxyethylamine (1-ephenamine penicillin G). The free base itself has been shown to possess anti-allergic properties apparently unrelated to true anti-histaminic action. Thus, administration of the penicillin salt is claimed to reduce

the incidence of sensitisation in patients receiving normal penicillin therapy. Cases are quoted in which patients who have reacted to treatment with procaine or sodium penicillin have responded well to the new preparation with subsidence of the reaction effects. This advantage of 1-ephenamine penicillin G and the fact that it produces a depot effect approximating to that of procaine penicillin—thereby lending itself to similar pharmaceutical presentations—suggest that it may have a place in the growing list of useful antibiotic preparations.

1 (c) *Preparations for systemic use—selective tissue concentration.*

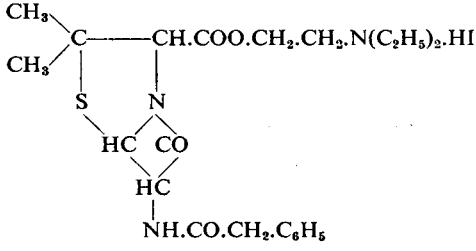
(i) *Penicillin esters and penethamate hydriodide.* Oil-soluble penicillin esters have also received some attention in the past as potential agents for delayed absorption therapy. The methyl, ethyl, *n*-butyl and benzhydryl esters were first prepared by Mayer, Hobby and Chaffee,[43] who showed that they were active in the infected mouse because of their hydrolysis in the animal's body to yield free penicillin. Other investigators[44,45,46] have shown, however, that only mice, rats and guinea-pigs were able to hydrolyse the esters of free penicillin, but that this was not possible to the dog, the rabbit, the monkey or man; it was therefore concluded that the esters of penicillin were of no therapeutic value.

Later, Carpenter[47] described the preparation of the dimethylaminoethyl ester hydrochloride of penicillin, which was readily hydrolysed *in vitro* in aqueous buffer at *p*H 7·3. More recently, Jensen *et al.*[48] synthesised a number of dialkylaminoethyl esters of benzylpenicillin and submitted them to detailed investigations *in vitro* and *in vivo* with the primary object of discovering new penicillin derivatives for delayed absorption therapy. The hydriodide of the diethyl ester—penicillin G diethylaminoethyl ester hydriodide—has since aroused widespread interest because, besides having a marked depot effect (though rather less than procaine penicillin), it was found to exhibit the remarkable property of concentrating penicillin in lung tissues, especially when these are inflamed. Although the concept of selective concentration of antibiotics in particular organs or tissues is not entirely new, this would seem to be the first product for which it has been shown to have practical significance in the treatment of disease.

(ii) *Mode of action of penethamate hydriodide.* Because of the complexity of the factors involved, the causes of the affinity of the ester for lung tissues are not known with certainty. Ungar and Muggleton[49] have thrown light on this aspect in a recent report describing the effects of the hydriodide in the laboratory animal. Among the tentative reasons advanced by them for the selective accumulation of the drug in lung tissues are: (1) quantitive influence of tissue esterase on the rate of hydrolysis; (2) prolongation effect from the antidiuretic action of diethylaminoethanol; (3) physico-chemical interaction between the ester and lung tissue (e.g., binding on tissue proteins); (4) influence of molecular size and structure; (5) differences in the chemical composition and cell constituents of tissue exudates. Discovery of the unique property of selective lung action in this ester hydriodide may well be a milestone in

medical progress. Although much remains to be done before its behaviour is properly understood, what is already established clearly points the way to future discoveries in a relatively unexplored field of research.

(iii) *Properties.* Benzylpenicillin 2-diethylaminoethyl ester hydriodide (penethamate hydriodide; estopen) occurs as a white crystalline powder with



a theoretical potency of 1058 I.U./mg. It is sparingly soluble in water (about 1 per cent. at 20° C.), a saturated solution having a *p*H of about 5·0. Aqueous solutions are unstable owing to hydrolysis of the penicillin ester to free penicillin and diethylaminoethanol, the velocity of the reaction increasing with rise in temperature and *p*H. Hydrolysis is pronounced above *p*H 7·0 and slowest between *p*H 4·0 and 5·0. The unhydrolysed ester is not affected by penicillinase but the free penicillin liberated by de-esterification in aqueous solution is inactivated by the enzyme.

(iv) *Formulation and stability.* As with procaine penicillin, satisfactory aqueous suspensions of penethamate hydriodide cannot be prepared in water alone. Foaming and entrainment of air and solid particles in the froth, as well as a tendency for unwetted particles to cling to the walls of the container, detract from pharmaceutical elegance and convenient administration from a hypodermic syringe. In this country the drug is issued in two strengths—100,000 and 500,000 I.U.—as finely divided sterile powders containing small quantities of wetting, suspending and buffering agents of similar composition to those used for procaine penicillin. On addition of water the powder is converted to a uniform suspension that can be withdrawn and injected with ease.

In dry form, penethamate hydriodide has a shelf life of at least 2 years. If stored in a cool place (below 25° C.) sterile aqueous suspensions retain their potency for a maximum of 7 days and for at least 30 days in a refrigerator (4° C.). Since experimental evidence[49] indicates that the phenomenon of selective lung concentration is dependent on the *intact* ester link within the molecule, it is important that hydrolysis be delayed as far as possible until the ester reaches the desired site. In order to ensure this happening it is essential to know the rate of hydrolysis in pharmaceutical suspensions. Some typical results are given in Tables III and IV being based on determinations of unchanged ester-hydriodide. After 7 days storage at 24° to 26° C. the total loss of penethamate hydriodide was 12·4 (9·9 to 14·9 for P = 0·95) per cent.; no loss occurred after 8 weeks storage at 4° C. Reduction in *p*H is accelerated by increase in temperature.

1 (d) *Preparations for systemic use—oral therapy.*

In the early days of penicillin, scarcity, expense and inadequate dosage, as well as the uncertain fate of the antibiotic in the alimentary tract, tended to discredit the value of oral therapy. Until the last year or so, dose schedules have been largely conditioned by amounts and costs. There has thus been a tendency to sub-optimal oral dosage with poor or indifferent clinical response. Thanks, however, to increased production and improved methods of manufacture, economic considerations

TABLE III

STABILITY OF AQUEOUS SUSPENSIONS OF PENETHAMATE HYDRIODIDE STORED AT 24° TO 26° C.

Determination	Percentage Potency Remaining After						
	1 Hour	3 Days	4 Days	6 Days	7 Days	10 Days	14 Days
Total potency per vial	(103) 100 (96·9)	—	(101·2) 98 (95)	—	(94·4) 92 (89·4)	(90·7) 87·6 (85·1)	(93·8) 89·4 (85·7)
Unhydrolysed ester-hydriodide remaining	(100) 96·9 (93·8)	—	(98·8) 95·7 (92·6)	—	(90·1) 87·6 (85·1)	(87) 83·9 (81·4)	(91·3) 87 (83·2)
*p*H	6·59	—	5·55	—	4·87	4·66	4·70

NOTE:—In this and Table IV the unhydrolysed ester remaining represents the difference between the total potency of the suspension and the filtered supernatant liquid. Bio-assays (20 plate) were used and fiducial limits (in brackets) are stated for P = 0·95. Approximate potency of suspension = 250,000 IU./ml.

TABLE IV

STABILITY OF AQUEOUS SUSPENSIONS OF PENETHAMATE HYDRIODIDE STORED AT 4° C.

Determination	Percentage Potency Remaining After						
	1 Hour	1 Week	2 Weeks	3 Weeks	6 Weeks	6 Weeks	8 Weeks
Total potency per vial	(103) 100 (96·9)	(105·6) 101·9 (97·5)	(103) 98·8 (94·4)	(98·8) 95·7 (92)	(105) 101·9 (98·8)	(109·3) 105·6 (102·5)	(109·3) 105·6 (102·5)
Unhydrolysed ester-hydriodide remaining	(101·2) 98·1 (95)	(103) 99·4 (95)	(100) 95·7 (91·3)	(95·7) 92·6 (88·8)	(102) 98·8 (95·7)	(105·6) 101·9 (98·8)	(105) 101·2 (98·1)
*p*H	7·37	6·00	6·11	5·96	5·76	5·86	5·85

now carry far less weight than they did 5 years ago. Moreover, despite the seemingly inevitable disparity between effective oral and parental dosage (a ratio of approximately 5 : 1), there is increasing appreciation of the fact that oral administration may be justified because of its extra convenience.

While oral therapy can never compete with aqueous injection of penicillin in the treatment of the more acute conditions, it is, none the less, a valuable method for treating early and localised infections. It is useful, too, for dealing with infants and young children whom the doctor wishes to spare the pain or psychological upset of injections. A further advantage is that it enables treatment to be carried out at home by the patient without medical attendance.

The growing interest in oral penicillin therapy has focussed attention on new formulations and methods of presentation, of which some of the more important are briefly considered below.

(i) *Tablets.* The most widely used presentation is the simple tablet made of the sodium or potassium salt of benzylpenicillin. Tablets of from 50,000 to 400,000 I.U. each are commercially available: the higher potencies—200,000 I.U. and above—are being increasingly used. The fact that it is traditional to describe penicillin dosage in terms of units, whereas oral dosage with the newer antibiotics is measured exclusively by weight, is confusing and can be misleading to prescribers in calculating the relative costs of treatment. In oral therapy there would seem to be a case for mentioning both weight and units when stating the dosage of penicillin.

The essential criteria for a stable and otherwise acceptable tablet are: (1) simplicity of formulation with exclusion of all other ingredients except for a trace of lubricant and, where necessary, an inert and non-hygroscopic filler: (2) control of moisture (preferably $\not>$ 0·5 per cent.): (3) rapid disintegration. Absorption of penicillin from the stomach is poor. Maximum utilisation occurs in the small intestine, notably in the duodenum, where inactivation is much less than in the jejunum or ileum.[50,51] A quick disintegration or solution rate (if the tablet is made from entirely soluble ingredients) of approximately 5 to 15 minutes would therefore seem desirable to ensure that the penicillin is in solution and available for absorption by the time it reaches the duodenum. In clinical studies with oral penicillin, Boger and Beatty[52] have stressed the importance of disintegration and warn against the combined dangers of excessive compression during manufacture of the tablets and an imperfectly balanced ratio of penicillin to excipient. Carefully formulated oral tablets packaged in moisture-proof containers, made with sodium or potassium benzylpenicillin will retain their potency under normal conditions of storage for at least 18 months.

Non-ionic surface active agents (e.g., polyoxyethylene sorbitan mono-oleate) have been administered orally in conjunction with penicillin to increase absorption in the upper intestine by improving dispersion and lowering surface tension.[53] Results, however, were disappointing.

Sparingly soluble penicillin salts—e.g., aluminium and procaine penicillin—have been investigated by American workers[52,54] to test the hypothesis that low solubility in water would protect against acid destruction during passage through the stomach and thereby give concentrations in the blood more prolonged than is usual with soluble penicillins. It was concluded that none of the insoluble salts administered by mouth were any more effective than sodium or potassium benzylpenicillin.

(ii) *Antacids, buffers and other forms of protection.* At one time there was a widely held belief that penicillin by mouth ran the distinct risk of being rendered substantially inactive by the acid gastric secretion. To-day, however, there is increasing appreciation that this is not a basically important factor, provided that the antibiotic is administered in sufficiently large doses and preferably on an empty stomach.

The earlier belief that protection against acid destruction was essential for effective oral therapy led to the introduction of numerous methods for combating inactivation. Many of these depended on the collateral administration of various antacids and buffer salts, all of which are too well known to require detailed mention here. Penicillin tablets buffered with sodium citrate have been commercially available for some time, but practical considerations of tablet size in relation to ease of swallowing precludes the incorporation of therapeutically effective quantities of buffer salt. An interesting development in this connection has been the introduction in America of an effervescent tablet claimed to contain 300 per cent. more buffering alkali than any comparable product.

An alternative approach was the administration of penicillin in oily suspensions, but wide variations in the rate of absorption of the antibiotic gave rise to poor clinical results. For similar reasons enteric coating has been found wanting by Florey and Florey[55] and by Brindle and Keepe.[56]

Although of doubtful practical utility, several ingenious methods of protecting penicillin against acid destruction during oral therapy have been the subject of patents. For example, ion-exchange resins have been used:[57] coating the finely divided antibiotic with waxes such as jojoba wax has been described:[58] dry porous tablets said to possess "enteric" properties have been prepared from hydrogenated vegetable oils and fats containing various percentages of alkali stearates, sodium oleate (as emulsifier), starch and crystalline potassium penicillin.[59]

(iii) *Liquid preparations—modern trend in presentation.* The soluble salts of penicillin are characterised by an unpleasant bitter taste extremely difficult to disguise in dry compressed products. The problem is not lessened by the current demand for higher dosage coupled with obligatory restriction of tablet size. Sugar coating, an obvious solution, presents practical difficulties in both stability and disintegration. Encapsulation in gelatine is less economical than tabletting and cannot always be relied upon to provide the same rapidity of disintegration as careful formulation of a tablet. A further point is that tablets—by means of cursor or cleavage lines—lend themselves to the administration of divided doses, whereas capsules do not.

It is true of unpleasant tasting drugs in general that liquid presentations afford the pharmacist maximum scope for achieving palatability. Further, pleasantly flavoured liquids are acceptable to infants and young children, who also find them easier to swallow. Recognition of this fact, coupled with the growing interest in oral penicillin, has encouraged the development of new liquid presentations, most of which are designed to facilitate mixture of the antibiotic with the vehicle at the time of dispensing.

Such preparations mostly contain crystalline sodium or potassium penicillin G, as a powder or as soluble tablets, and are supplied along with a bottle of an attractively flavoured diluent so compounded as effectively to mask the taste of the penicillin. Alternative formulations avoid the expense of dual containers by presenting the penicillin and all adjuvants in one bottle (as a dry mixture) with directions to the pharmacist to add

a specified quantity of water at the time of dispensing. Potencies range from 50,000 to 500,000 I.U. per teaspoonful and buffering agents are frequently incorporated, presumably for pharmaceutical rather than therapeutic reasons. Needless to say, the life of dispensed solutions is restricted to seven days, during which they must be stored in a refrigerator. It therefore follows that these preparations can only enjoy widespread application in countries such as the United States, where practically all members of the community have easy access to one.

There is little doubt that the most important advance of recent years in this field has stemmed from the development of new insoluble salts of penicillin, such as *N,N'*-dibenzylethylenediamine dipenicillin G. The combined advantages of low solubility, stability and absence of taste enable this salt to be presented as a palatable ready-to-use oral suspension that is said to be stable for 18 months at normal temperatures. With it penicillin concentrations in the blood are stated to be closely similar to those produced by similar oral doses of procaine or potassium salts of penicillin.[41] The all-round merit of such preparations suggests that they are likely to have an important influence on the future of oral penicillin therapy. They also provide an excellent example of the part played by the chemist in modifying the physico-chemical properties of a drug and so enabling the pharmacist to present it in a more acceptable and stabler form.

Oral doses of penicillin in combination with one or more of the sulphonamides are sometimes used in the treatment of mild streptococcal, pneumococcal or gonococcal infections. They are usually presented as tablets or occasionally as dry flavoured powders to be reconstituted with water by the dispenser when needed for use.

2. *Preparations for local therapy.*

Penicillin preparations for topical application are too numerous to be considered in any detail here. They range from "micronised" powders for inhalation therapy to antibacterial toothpowders, with diverse intermediate examples. Some have incurred severe criticism—particularly in the United States—because of the tendency to produce sensitisation, which is especially prone to occur when, for instance, an ointment or lotion contains only a small amount of penicillin and treatment is unnecessarily prolonged.

Of the numerous formulations described in the literature many are inevitably of limited interest to the industrial pharmacist, if only because they do not lend themselves to presentation in forms stable enough to withstand long periods of storage under varied climatic conditions. Even the official lozenge and ointment have given rise to serious problems of stability, the solution of which has been made possible by changes recently sanctioned in the Addendum 1951 to the British Pharmacopœia, 1948. The implications of these changes are discussed below.

(i) *Lozenges.* The British Pharmacopœia, 1948, prescribed the use of calcium penicillin for making lozenges. It also indicated the nature of the base to be used and defined the conditions of storage (in air-tight

containers below 15° C.). Attention has already been drawn to the instability of calcium penicillin lozenges stored at elevated temperatures[60] and to the marked variations among products of different manufacture.[61] Later, lozenges manufactured from a ball-milled triturate of penicillin (calcium salt) and magnesium stearate were reported to be stable for twelve months at ordinary room temperature.[62] No results were given for storage at higher temperatures.

Considerable additional experience in our laboratories, including an investigation of calcium penicillin lozenges issued by other manufacturers, led to the following conclusions:—

1. At ordinary room temperature, some products retained their potency much better than others: generally, however, potency tended to decline steadily with increase of time: instability could not be attributed to any differences in moisture content.

2. At higher temperatures (38° C.) loss of potency was general and substantial: some products lost more than 50 per cent. of their initial activity in eight months.

3. Various methods of manufacture (including ball-milling with stearates) were tried: none revealed any correlation between loss of potency and technique of processing.

4. Triturates of the penicillin in pre-dried lactose, starch, tragacanth or magnesium stearate were substantially stable at atmosphere temperatures, but losses similar to those observed with lozenges occurred at 38° C. Sucrose occasionally gave rise to indifferent results and its use is still undergoing investigation.

5. Marked variations in stability were observed with lozenges made from different batches of calcium salt of identical origin. Similar variations were noted when calcium salts of different manufacture were compared.

To sum up: unsatisfactory stability of the original lozenge of penicillin is attributed mainly to inherent weaknesses of the calcium salt, namely, (*a*) variable composition and (*b*) lack of stability to heat, for which there is independent confirmation.[4] In general, for all low potency preparations of penicillin the control of moisture (preferably $\not> 0{\cdot}5$ per cent.) is important. However, so far as our experience has gone, moisture variation below an upper limit of 1 per cent. is not a factor influencing stability.

The revised monograph on penicillin lozenge in the Addendum 1951 to the British Pharmacopœia, 1948, sanctions the alternative use of benzylpenicillin (sodium or potassium salt). Lozenges made with these salts have the following advantages: (*a*) superior stability at elevated temperatures, with consequent avoidance of the need to store below 15° C. and facilitated distribution in hot climates: (*b*) less penicillin overage required to compensate for losses during storage. Yet another advantage, now widely recognised, is that the crystalline sodium or potassium salts of penicillin are less hygroscopic than any of the amorphous salts. Preliminary observations[60] suggested that the reverse was true of calcium penicillin, but radical changes in the primary

manufacture of the crystalline sodium salt and further experience have refuted this. The advantage is, however, not sufficient to discount the necessity for protecting the lozenges against attack from moisture during storage.

Table V illustrates a comparison between lozenges (theoretical potency 2000 I.U./lozenge) made with calcium penicillin and crystalline sodium penicillin G. A base of sucrose and gum was used for both, and the lozenges were stored in bottles with screw caps having waxed cardboard liners.

TABLE V

STABILITY OF PENICILLIN LOZENGES MADE WITH CRYSTALLINE AND AMORPHOUS CALCIUM PENICILLIN 2000 I.U./LOZENGE

Lozenge	Moisture per cent.	Temperature	Percentage Potency Remaining After: Months						
			0	1	2	4	6	8	10
Crystalline Sodium Penicillin	0·98	Room	100	100·5	106·5	97·6	—	105	100
		38° C	100	105	106	92·5	102	—	96
Amorphous Calcium Penicillin	0·88	Room	100	102	103	90·5	—	87	77·5
		38° C	100	89·5	76	71·5	58	—	49

(ii) *Ointments.* In a preliminary communication,[60] attention has been drawn to the effect of wool alcohols on the stability of penicillin ointments. Further studies have confirmed the adverse effect that this ingredient can have on the stability of penicillin. Hard paraffin, though less damaging than wool alcohols, is also contra-indicated. Carbowaxes (high molecular weight polyethylene glycols), cetyl-stearyl alcohol, self-emulsifying stearyl alcohol, cocoa butter, lanette wax "SX", polychols (water soluble polyoxyethylene condensation product of wool wax alcohols) and many ionic and non-ionic surface-active agents are also destructive to the antibiotic.

In the Addendum 1951 to the British Pharmacopœia the composition of the base for the skin ointment has been changed to a mixture of liquid and soft paraffins, in which penicillin is much more stable, especially at elevated temperatures. Further, since the initial moisture content of the old and the new base is so low that the variations occurring in practice are not critical for stability, the earlier injunction to pre-heat the base at 110° C. has been deleted. The new monograph also permits the use of crystalline sodium or potassium salts of penicillin as alternatives to the amorphous calcium or potassium salts in the ointment. As in the lozenge the former salts give greater stability under adverse conditions of storage, with concomitant reduction in overage.

Tables VI and VII show some typical results for ointments (500 I.U./g.) under various conditions of storage.

It is evident from these results that the best method of presenting penicillin ointment is in collapsible tubes. Deterioration in pots and Petri dishes was noticeably greater, with little to choose between them. The increases in moisture content were surprisingly small and there is

little evidence to connect them with loss in potency of samples stored in pots and Petri dishes.

Permissive use of benzylpenicillin (sodium or potassium salt) is also extended to the ophthalmic ointment of penicillin. However, the base is unchanged, despite the fact that several workers have drawn attention to the detrimental effect of lanolin and crude cholesterinated bases in general on the stability of penicillin.[63,64,65]

TABLE VI

STABILITY OF PENICILLIN OINTMENT (B.P. 1948) IN TUBES, POTS AND PETRI DISHES

Storage	Batch No. 1			Batch No. 2		
	Percentage Loss (6 months)	Moisture (per cent.)		Percentage Loss (6 months)	Moisture (per cent.)	
		Initial	Final		Initial	Final
In Tubes ..	43	0·03	0·04	34	0·03	0·05
In Pots	51	0·03	0·03	46	0·03	0·08
In Petri dishes ..	56	0·03	0·04	46	0·03	0·08

TABLE VII

STABILITY OF PENICILLIN OINTMENT (SIMPLE PARAFFIN BASE) IN TUBES, POTS AND PETRI DISHES

Storage	Calcium Penicillin			Penicillin G Sodium		
	Percentage Loss (6 months)	Moisture (per cent.)		Percentage Loss (6 months)	Moisture (per cent.)	
		Initial	Final		Initial	Final
In Tubes ..	25	0·02	0·05	8	0·02	0·05
In Pots	48	0·02	0·04	22	0·02	0·12
In dishes Petri ..	35	0·02	0·10	20	0·02	0·07

(iii) *Penicillin creams.* Antibacterial creams formulated with oil-in-water emulsified bases are sometimes preferred to ointments because of their superior æsthetic and medical qualities. There are two examples of such creams in the British Pharmacopœia. Both, however, suffer from the disadvantage of very limited stability (only storage in the refrigerator being allowed) because of the rapid decomposition of the soluble penicillin salts used in their preparation.

An interesting development in this connection has been the recent introduction of a stabilised high potency cream of procaine penicillin (10,000 I.U./g.) containing some 50 per cent. of water and possessing a shelf-life of 12 months at room temperature.

STREPTOMYCIN AND DIHYDROSTREPTOMYCIN

The discovery of streptomycin arose from a carefully planned search by Waksman and his collaborators in 1939 for an antibiotic that would bring about inhibition and lysis of Gram-negative bacteria. After having isolated and examined several thousand actinomycetes, these

workers produced a substance called streptothricin. It proved to be too toxic for general use and shortly after there followed the isolation of streptomycin from *Streptomyces griseus*.[66] To-day, streptomycin is produced in large quantities by deep fermentation, as is penicillin. Dihydrostreptomycin is prepared by hydrogenation of streptomycin in presence of a platinum catalyst.

The Addendum, 1951, to the British Pharmacopœia, 1948, contains monographs on four compounds of streptomycin, namely, streptomycin-calcium chloride, streptomycin hydrochloride, streptomycin sulphate and dihydrostreptomycin, either as the hydrochloride or the sulphate. There are also separate monographs for injectable preparations of each compound. Because one unit of antibacterial activity is approximately equal to 1 μg. of streptomycin base, the unit has been re-defined as such in the United States, where potencies are normally expressed in terms of micrograms of base in 1 mg. of product. In contrast, the British Pharmacopœia adheres to the original method of defining potencies in terms of units. It will also be noted that a biological assay is mandatory in this country, mainly as a safeguard against possible contamination of streptomycin with mannosidostreptomycin (streptomycin B), which is considerably less active against most micro-organisms.

In its antibacterial activity streptomycin is effective against a wide range of organisms, both Gram-negative and Gram-positive. For treating the latter, however, penicillin is usually more effective and is preferred because of its freedom from toxicity. Broadly speaking, therefore, streptomycin is complementary to penicillin in combating infections due to Gram-negative organisms. The outstanding use of streptomycin to-day is in the battle against tuberculosis—especially miliary tuberculosis and tuberculous meningitis—against which it has proved a most valuable adjunct to previous medical treatment.

1. *Preparations for systemic treatment.*

Under this heading it will be convenient to deal collectively with the injectable preparations of streptomycin, because they are similar in presentation, formulation and stability.

(i) *Presentation.* Like soluble penicillin, streptomycin and dihydrostreptomycin are normally presented in rubber capped vials as amorphous or crystalline solids. Vials usually contain sufficient to provide the equivalent of 1 g. of streptomycin base for conversion to solution with sterile water as required. Except for very dilute solutions, saline solution is contra-indicated as a solvent because of the risk of irritation from excessive hypertonicity. Even with 250,000 I.U./ml., the official strength for intramuscular injections, solutions contain approximately 30 per cent. w/v of solids.

Stabilised aqueous solutions of dihydrostreptomycin sulphate are available in some parts of the world. These are said to retain their potency for 12 months at ordinary temperatures. Several contain procaine penicillin as well as dihydrostreptomycin, thereby giving a widened range of antibacterial activity.

Dry mixtures of streptomycin or dihydrostreptomycin, or both, with penicillin (sodium or procaine salts or both), for extemporaneous dispensing, are also coming into use. They provide in one injection a "broad spectrum" antibiotic preparation exploiting a useful synergistic action against mixed infections. Formulation follows the same pattern as that described for procaine penicillin in aqueous suspension.

Penetration of streptomycin into the cerebrospinal fluid is poor, so that in meningitis normal systemic treatment must be supplemented by intrathecal injection. In view of the need in such conditions for extreme precautions about purity, clarity and sterility, the most satisfactory presentation would seem to be a single-dose ampoule. Systemic treatment by mouth is impracticable, because streptomycin is poorly absorbed from the gastro-intestinal tract. Oral therapy is therefore confined to the treatment of intestinal infection, against which it is sometimes used to supplement injections.

In the present-day treatment of tuberculosis, injections of streptomycin are frequently augmented by oral administration of *p*-aminosalicylic acid, usually in daily doses of 12 to 18 g. It is claimed that it has a synergistic effect and also that by its use the onset of streptomycin resistance is delayed.

(ii) *Stability.* Provided they are stored in a cool dry place, the official salts of streptomycin and dihydrostreptomycin are stable for at least 2 years.

In general, dilute aqueous solutions (25,000 to 50,000 I.U./ml.) remain substantially stable over long periods of time, even at 38° C. Development of colour, however, increases with rise in temperature. Strong solutions (250,000 to 500,000 I.U./ml.) also retain their potency for considerable periods in the refrigerator and at room temperature, but they are less stable at 38° C. Discolouration is more rapid in strong solutions and increases markedly with rise in temperature. There is also a tendency for solutions to deposit when stored for excessive periods. Storage in a refrigerator retards the development of colour.

The discolouration of streptomycin solutions does not appear to be related to loss of antibacterial activity, nor is there any evidence that coloured solutions are more toxic than freshly prepared ones. Nevertheless, it is an undesirable quality, accentuated by the fact that streptomycin salts from different manufacturers can vary in their propensity to develop colour. Although the answer will probably be found in improved methods of manufacture, it is questionable whether there was justification at the time of publication of the Addendum, 1951, to the British Pharmacopœia, 1948, for the liberal storage period of 1 month at room temperature.

It is not known with certainty how critical is the relationship between *p*H and the stability of streptomycin, although it is generally held that solutions are stable between limits of *p*H 3 and 7. Tentative evidence suggests that *p*H 7·0 or even slightly higher is advantageous, especially for dihydrostreptomycin, but more work is required to check this.

(iii) *Bacteriostatics.* Chlorocresol, chlorbutol, phenol and phenylmercuric nitrate, in the customary concentrations, are satisfactory preservatives for solutions of streptomycin. The suitability of three of these for use with streptomycin-calcium chloride has been confirmed by Rolph and Usher.[67]

2. *Preparations for local therapy.*

Although the primary use of streptomycin is in the treatment of tuberculosis, the fact that it is effective against a wide range of Gram-negative organisms has led to the introduction of various preparations for topical application. Some depend on the use of streptomycin alone; others reflect the growing interest in combined antibiotics to achieve the advantage of additive or even synergistic action in the treatment of mixed infections.

In formulating anhydrous preparations—e.g., ointments and powders—the sulphates of dihydrostreptomycin or streptomycin are preferred to the calcium chloride complex salt, which has the disadvantage of being hygroscopic. For other local uses there is little to choose between the salts, except that a sensitive person might possibly experience less irritation from eye drops prepared with one or other of the sulphates rather than with the calcium chloride complex.

The successful combined parenteral application of penicillin and dihydrostreptomycin has led to their similar use in an ointment for treating mixed infections in wounds, indolent ulcers and certain superficial skin conditions. As yet few preparations of streptomycin for local application have appeared on the market in this country. In America, however, their use is increasing as seen by the revised index to the U.S. Federal Register (Food and Drug Administration), from which the following examples are taken:—penicillin-streptomycin-bacitracin ointment: streptomycin and dihydrostreptomycin tablets: streptomycin-polymyxin-bacitracin tablets: dihydrostreptomycin and kaolin in gel: streptomycin otic with antifungal agent.

New Antibiotics

The discovery of streptomycin focussed attention on the antimicrobial properties of the actinomycetes as a potential source of other antibiotics. As a result of extended investigations into this group of micro-organisms, several new substances have been isolated in the United States, among the more important of them being chloramphenicol, aureomycin and terramycin. It has become common practice to refer collectively to these and similar new chemotherapeutic agents as the "wide-spectrum" antibiotics, because they show activity against a wider range of micro-organisms than does penicillin alone. They are of undoubted value in the treatment of specific infections that do not respond to penicillin—for example, virus pneumonia and typhoid fever—but their indiscriminate use can produce serious toxic reactions. In this respect they differ from penicillin, which, besides having numerous clinical applications, is unique in being practically free from all side-effects.

CHLORAMPHENICOL

During 1947, chloramphenicol, originally called chloromycetin, was isolated from the culture fluid of a species of *Streptomyces* discovered in soil from Caracas, Venezuela.[76] It was independently isolated from a *Streptomyces* found in a compost heap at the Illinois Agricultural Experimental Station. Chloramphenicol is to-day the only synthetically prepared antibiotic. It is D(−)-*threo*-2-dichloroacetamido-1-*p*-nitrophenyl-1:3-propanediol. It is a bitter tasting white or off-white crystalline compound, sparingly soluble in water (2·5 mg./ml. at 25° C.), but readily soluble in ethanol, propylene glycol and acetyldimethylamine. In the dry state the antibiotic is stable for long periods at ordinary temperatures. Acid and neutral solutions are stable: in alkaline solution activity is lost owing to hydrolysis at the amide linkage. Between *p*H 2 and *p*H 6 solutions may be boiled for an hour with little loss of potency. Greater loss occurs on autoclaving (20 minutes at 120° C.). Below *p*H 2 or above *p*H 6 rapid loss of potency occurs on boiling or autoclaving.[77]

1 (a) *Preparations for systemic therapy—oral.* Chloramphenicol is active when administered orally. Other routes of administration are used only when high blood levels are required quickly or when a patient is unable to take the drug by mouth. Hard gelatin capsules containing 50 or 100 mg. are available. For children, however, a comparatively tasteless ester—chloramphenicol palmitate—has been specially developed.[78] A suspension of the palmitate in a flavoured vehicle is reputed to taste like custard! It contains in 1 ml. the equivalent of 31 mg. of chloramphenicol and has *p*H 6 to 7. The product is stable for 12 months at room temperature. Absorption is slightly slower with this preparation, because the ester must be hydrolysed in the alimentary tract before the chloramphenicol can be absorbed.

1 (b) *Preparations for systemic therapy—parenteral.* The low solubility of chloramphenicol in water complicates the preparation of parenteral dose forms. However, solutions in acetyldimethylamine may be diluted with water, saline solution or dextrose solution without precipitation.[79,80,81] Care must be taken to add the antibiotic solution to the diluent and below its surface. Ampoules (2 ml.) containing a 25 per cent. solution of chloramphenicol in 50 per cent. aqueous acetyldimethylamine are available for intravenous injection, diluted or undiluted. Such solutions are said to be stable for 12 months at room temperature.

2. *Preparations for local therapy.* Pure powdered chloramphenicol has been applied to ulcers and placed in operation wounds; dusting powders (500 to 1000 mg. per ounce) made up with lactose, or with equal parts of starch and zinc oxide, have been applied to larger surface wounds.[82]

Chloramphenicol is also used in ointments and creams containing about 1 per cent. of the drug. For ear infections a 10 per cent. solution in propylene glycol is used; eye drops are prepared extemporaneously by addition of water to a dry mixture of the drug and a borate buffer.

AUREOMYCIN

The isolation of aureomycin from the culture fluid of *Streptomyces aureofaciens* was reported by Duggar[68] in 1948. Like terramycin, aureomycin is an amphoteric substance: it is fairly soluble in water at both high and low *p*H, but sparingly soluble near the neutral point; above *p*H 8·5 it is readily soluble. The base is soluble in water to the extent of 0·5 to 0·6 mg./ml. at 25° C. The hydrochloride is more soluble (about 14 mg./ml. at 25° C.) to give a solution with *p*H 2·9. Provided it is stored in well-closed containers protected from light, dry crystalline aureomycin hydrochloride is stable at normal temperatures. It is fairly stable in acid solution, but is rapidly inactivated above *p*H 7.[69] A 1 per cent. solution may be kept frozen for 6 weeks without loss.[70] The polysaccharide fraction of egg yolk prolongs the life of aqueous solutions[71] and has been suggested for preventing deterioration of aureomycin during biological assay.

1 (a). *Preparations for systemic use—oral.* Aureomycin hydrochloride (now included in the British Pharmaceutical Codex by the 1952 Supplement) is customarily administered by mouth in hard gelatin capsules containing 50 or 250 mg. of the drug. Anorexia, nausea and vomiting are common sequelæ; to prevent them all the well known antacids and buffering agents have been tried. Some of these relieve the symptoms by simply reducing absorption of the aureomycin. For example, aluminium hydroxide gels have been shown to adsorb both aureomycin and terramycin hydrochlorides in amounts ranging from 25 to 90 per cent.[72,73] Bismuth subsalicylate was free from this disadvantage. A more recent report[74] describes the successful use of tablets incorporating the antibiotic and antacid in the same preparation. Aureomycin calcium caseinate was combined with calcium caseinate and calcium carbonate.

1 (b). *Preparations for systemic use—parenteral.* In exceptional cases it may be desirable to administer aureomycin intravenously. A leucine buffer was formerly employed for this purpose, but has since been replaced by sodium glycinate because of the tendency of the former to cause thrombophlebitis.[70] The *p*H of the buffered solution is about 8 to 9. Intramuscular injections cause local pain and irritation and are not used. Although aureomycin passes the blood-brain barrier when the meninges are inflamed, it may not always do so in sufficient amount to control the infection. A solution suitable for intrathecal use may be prepared by mixing aseptically immediately before use 1 ml. of a 1 per cent. solution of aureomycin hydrochloride with 9 ml. of a specially prepared solution of sodium glycinate.[70] The final solution has a *p*H of 7·2 to 7·4. It may be cloudy owing to precipitated aureomycin, but apparently this is without adverse effect.

2. *Preparations for local therapy.* Lozenges containing 15 mg. of aureomycin hydrochloride are used to supplement oral systemic therapy in the treatment of mouth and throat infections. Ophthalmic drops are prepared by adding water to a mixture of the hydrochloride, sodium borate (buffer) and sufficient sodium chloride to render the solution isotonic.

Solutions should be recently prepared (according to U.S. Federal Register they are stable for 2 days in a refrigerator). Ear drops in a 5 per cent. solution of benzocaine in propylene glycol are stated to be stable for 7 days at 15° C.

For skin infections, 3 per cent. aureomycin hydrochloride in soft paraffin or "carbowax" bases has been used with success.[75] An ointment containing 30 mg./g. is available commercially. Dental cones, dental paste, suppositories, surgical powder and gauze packing are commercially available in the United States.

TERRAMYCIN

Early in 1950 a new chemotherapeutic agent was introduced in the United States. It was called terramycin after *Streptomyces rimosus*, the soil organism[83] producing it. Terramycin is available as the crystalline base or as the hydrochloride.

Like aureomycin, terramycin is an amphoteric substance: the hydrochloride and disodium terramycin are both well defined salts. The base is not very soluble in water, but its solubility increases with fall or rise of *p*H owing to formation of acid or basic salts, respectively. Reasonably concentrated solutions are formed only at low and fairly high *p*H.[85,84] In the dry state, both the base and the hydrochloride are stable for long periods at room temperature. Even at 50° C. the hydrochloride loses only 5 per cent. of its potency after 4 months. The base shows no loss of potency after heating for 4 hours at 100° C. Disodium terramycin is less stable and darkens in colour at room temperature. Under these conditions it loses about 45 per cent. of its potency after 150 days. Stability in aqueous solution is largely a function of *p*H. Terramycin is most stable in acid solutions; at *p*H 1·0 to 2·5 solutions are stable for at least 30 days at 25° C. Alkaline solutions are less stable.

1 (a). *Preparations for systemic therapy—oral.* Terramycin is well absorbed when given by mouth, usually in capsules or as a solution or suspension in a flavoured vehicle. Capsules of 50, 100 and 250 mg. of the hydrochloride are available. A proprietary elixir containing 250 mg./5 ml. (as hydrochloride), in a buffered diluent with 20 per cent. of ethanol, has a *p*H of about 2·5 and a life of 2 weeks at room temperature. Lowering the alcohol content or raising the *p*H causes precipitation of terramycin with decreased stability.[86] A more concentrated form of a similar product is available for administration to infants as drops. An oral suspension of the base is also provided, with the same potency as the elixir but containing no alcohol. The *p*H is 5·6, which reduces the period of storage to one week.

1 (b). *Preparations for systemic therapy—parenteral.* The fact that therapeutically acceptable concentrations of terramycin can only be obtained in strongly acid or alkaline solutions creates obvious difficulties in making simple preparations for injection. Although acid solutions are irritant and, conversely, alkaline solutions are unstable, nevertheless a method of preparing a satisfactory intravenous injection of terramycin has recently been described.[87] It follows the same pattern as that for

aureomycin, sodium glycinate being used as a buffer: 9 parts of a special parenteral grade of the latter are mixed with 10 of terramycin hydrochloride. Addition of water gives a clear solution of *p*H 9·0 to 9·25, containing 100 mg. of terramycin activity and suitable for intravenous use. The dry mixture is stable over long periods. The prepared injection lost about 8 per cent. of its initial activity in 24 hours at room temperature, as well as after 72 hours at 5° C. Solutions darkened progressively during storage, owing to slow oxidation.

2. *Preparations for local therapy.* As with aureomycin, ophthalmic solutions (about 0·5 per cent.) are prepared extemporaneously from dry mixtures of terramycin, sodium borate and sodium chloride. The resultant solutions are said to be stable for 2 days in the refrigerator. An eye ointment containing 0·5 per cent. of terramycin in a soft paraffin base has been available for some time. Recently, stable sugar-coated tablets of terramycin base have been introduced, with the same potencies as capsules.

Antibiotic Therapy in Veterinary Medicine

This survey would be incomplete without at least a brief reference to the impact of antibiotics on the pharmacy of veterinary preparations. Apart from the use in veterinary practice of many of the established antibiotic preparations, special formulations and methods of presentation have been called for to suit particular purposes. Two products of current interest will suffice as practical examples; both of them reflect the influence of antibiotics on national economy. First, the importance of the problem of bovine mastitis to the dairy-farming industry and the success achieved with intra-mammary injections of penicillin and other antibiotics in controlling and eradicating the disease have called for the development of effective dosage forms in collapsible tubes with specially designed nozzles to facilitate convenient administration: formulation of improved bases and their evaluation *in vitro* and *in vivo* with different penicillin salts has been needed: the respective merits of presentation in collapsible tubes and bougies was also studied. A second, and equally interesting, development is well illustrated by the growing importance attached to antibiotics in nutrition. The observation that the addition of small quantities of an antibiotic to animal foodstuffs can induce measurable increases in growth—especially in pigs and table poultry—has aroused widespread interest. Although the study of antibiotics in nutrition is still in a preliminary stage, present evidence already indicates a promising future. Among the pharmaceutical problems thereby involved are: (*a*) selection of suitable carriers for the antibiotic with special reference to physical properties and stability under adverse conditions of storage over long periods: (*b*) conditions for stability over short periods after blending with various animal feedstuffs.

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