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 ($> 0{\cdot}03$ 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.*[61]

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.*

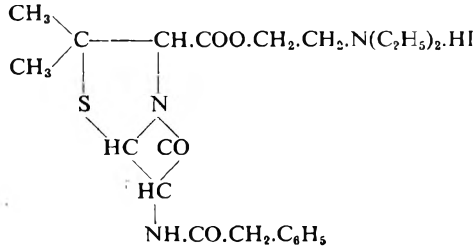
(i) *Penicillin esters and penethamate hydriodide.* Oil-soluble penicillin esters have also received some attention in the past as potential agents for delayed absorption therapy. The methyl, ethyl, *n*-butyl and benzhydryl esters were first prepared by Mayer, Hobby and Chaffee,[43] who showed that they 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. Unger 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)	(102·3) 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 < 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 $\not>$ 0·5 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	0·38	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,[50] 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.

REFERENCES

1. Monthly Digest of Statistics (H.M.S.O.).
2. Benedict, Schmidt, Coghill and Oleson, *J. Bact.*, 1945, **49**, 85; 1946, **51**, 291.
3. Pulvertaft and Yudkin, *Lancet*, 1946, **251**, 265.
4. Johnson and Lerrigo, *Quart. J. Pharm. Pharmacol*, 1947, **20**, 182.
5. Hahn, *Lancet*, 1947, **252**, 408.
6. Pratt, *Nature, Lond.*, 1947, **159**, 233.
7. Paul, Gaillot and Baget, *Ann. Pharm. franç.*, 1948, **8**, 524.
8. Macek, Hanus and Feller, *J. Amer. pharm. Ass., Sci. Ed.*, 1948, **37**, 322.
9. Clapham, *Pharm. J.*, 1950, **165**, 126.
10. Pederson-Bjergaard and Tonneson, *Svensk. farm. Tidskr.*, 1951, **55**, 239.
11. Ulex, *Arzneimittel-Forsch.*, 1951, **1**, 178.
12. Hadgraft, Hopper and Short, *Pharm. J.*, 1951, **167**, 13.
13. Carr and Wing, *Pharm. J.*, 1951, **167**, 63.
14. Hobbs, Livingstone, Reece and Woodard, *J. Pharm. Pharmacol*, 1952, **4**, 911.
15. Lester Smith, *Quart. J. Pharm. Pharmacol*, 1946, **19**, 309.
16. *Brit. Pat.*, No. 638, 535.
17. Swallow, *Pharm. J.*, 1952, **168**, 467.
18. Coulthard, Fawcett, Lewis and Sykes, *J. Pharm. Pharmacol.*, 1951, **3**, 748.
19. Kern, Terrill, Mann and Jones, *Science*, 1950, **112**, 787.
20. Romansky and Rittman, *Science*, 1944, **100**, 196.
21. Herrell, Nichols and Heilman, *Proc. Mayo Clin.*, 1947 (Dec. 10), **22**, 567.
22. *French Pat.*, No. 966, 263.
23. *U.S. Pat.*, No. 2, 544, 272.
24. Buckwalter and Dickison, *J. Amer. pharm. Ass.*, 1948, **37**, 472.
25. Thomas *et. al.*, *J. Amer. med. Ass.*, 1948, **137**, 1517.
26. Robinson *et. al.*, *J. Lab. clin. Med.*, 1948, **33**, 1232.
27. Kitchen, Thomas and Keen, *J. invest. Derm.*, 1949, **12**, 111.
28. Floyd, *Quart. J. Pharm. Pharmacol*, 1949, **1**, 747.
29. Romansky and Dowling, *J. Amer. med. Ass.*, 1947, **135**, 567.
30. Sullivan *et. al.*, *Science*, 1948, **107**, 169.
31. *U.S. Pat.*, No. 2, 507, 193.
32. Eastland, *J. Pharm. Pharmacol.*, 1951, **3**, 942.
33. McRoberts and Schulman, *Nature, Lond.*, 1948, **162**, 10[illegible].
34. Rideal, *Nature, Lond.*, 1949, **163**, 665.
35. Welch, Randall, Price and Hendricks, *Antibiotics and Chemotherapy*, 1951, **1**, 245.
36. *Brit. Pat.*, No. 658, 467.
37. *U.S. Pat.*, No. 2, 504, 482.
38. Monash, *Science*, 1947, **106**, 370.
39. Szabo, *et. al.*, *Antibiotics and Chemotherapy*, 1951, **1**, 494.
40. Seifter *et al.*, *ibid.*, 1951, **1**, 499.
41. Elias *et al.*, *ibid.*, 1951, **1**, 487.
42. Longacre, *ibid.*, 1951, **1**, 223.
43. Mayer, Hobby and Chaffee, *Science*, 1943, **97**, 205.
44. Richardson *et al.*, *Proc. Soc. exp. Biol. N.Y.*, 1945, **60**, 272.
45. Ungar, *Brit. J. exp. Path.*, 1947, **28**, 88.
46. Broh-Kahn and Smith, *Proc. Soc. exp. Biol., N.Y.*, 1946, **61**, 216.
47. Carpenter, *J. Amer. chem. Soc.*, 1948, **70**, 2964.
48. Jensen, Dragsted and Inter Kiuer, *Ugeskrift for Laeger*, 1950, **112**, 1075.
49. Ungar and Muggleton, *Brit. med. J.*, 1952, **1**, 1211.
50. Seeberg, Illg and Brown, *Science*, 1946, **104**, 342.
51. Seeberg, Illg and Brown, *J. Amer. pharm. Ass.*, 1946, **35**, 280.
52. Boger and Beatty, *J. invest. Derm.*, 1950, **15**, 373.
53. Schwartz and Boger, *J. Lab. clin. Med.*, 1949, **34**, 1443.
54. Boger, Beatty and Flippin, *Trans. Stud. Coll. Phys. Philad.*, 1949, **17**, 105.
55. Florey and Florey, *Lancet*, 1943, **244**, 387.
56. Brindle and Keepe, *Pharm. J.*, 1949, **163**, 266.
57. *Brit. Pat.*, No. 665, 073.
58. *U.S. Pat.*, No. 2, 487, 336.
59. *U.S. Pat.*, No. 2, 566, 200.
60. Woodard, *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 197.
61. Foster, *ibid.*, 1947, **20**, 403.
62. Barnard and Hartley, *ibid.*, 1948, **21**, 228.
63. Macek, Gakenheimer and Daughenbaugh, *J. invest. Derm.*, 1947, **8**, 209.

64. Tabillon and Murat, *Prod. pharm.*, 1948, **3**, 300.
65. Diding and Sandell, *Svensk. farm. Tidskr.*, 1949, **53**, 617.
66. Schatz, Bugie and Waksman, *Proc. Soc. exp. Biol., N.Y.*, 1944, **55**, 66.
67. Rolph and Usher, *Pharm. J.*, 1949, **163**, 494.
68. Duggar, *Ann. N.Y. Acad. Sci.*, 1949, **51**, 177.
69. Price, Randall and Welch, *ibid.*, 1948, **51**, 211.
70. Ainley, Walker and Bosanquet, *Lancet.*, 1952, **262**, 433.
71. Womack, Kass and Finland, *J. Lab. clin. Med.*, 1950, **36**, 655.
72. Waisbren and Hueckel, *Proc. Soc. exp. Biol., N.Y.*, 1950, **73**, 73.
73. Paul and Harrington, *J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 50.
74. Manning and Wellman, *Proc. Mayo Clin.*, 1952, **27**, 89.
75. *Press. Med.*, 1951, **59**, 542.
76. Erlich, Bartz *et al.*, *Science*, 1947, **106**, 417.
77. Trolle-Lassen, *Arch. Pharm. Chem.*, 1951, **58**, 780.
78. Glazko, Edgerton, Dill and Lenz, *Antibiotics and Chemotherapy*, 1952, **2**, 234.
79. *Brit. Pat.*, 671, 023.
80. Orr, Preisser *et al.*, *Antibiotics and Chemotherapy*, 1951, **1**, 63.
81. Payne, Bellerive *et al.*, *ibid.*, 1951, **1**, 138.
82. Coppleson, *Lancet*, 1951, **261**, 65.
83. Finlay, Hobby *et al.*, *Science*, 1950, **111**, 85.
84. Regna and Solomons, *Ann. N.Y. Acad. Sci.*, 1950, **53**, 229.
85. *Merck Index*, 6th Edition, 1952.
86. Fielitz and Leuallen, *Amer. prof. Pharm.*, 1951, **17**, 100.
87. Armstrong, Jarowski and Salivar, *J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 1.

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REPORT OF A SYMPOSIUM ON RECENT DEVELOPMENTS IN THE PHARMACY OF ANTIBIOTICS

AT the Symposium Session the Chairman, Mr. H. B. Mackie, presided, and introductory addresses were given by Mr. W. A. Woodard, Mr. W. Trillwood and Mr. J. O. Davidson.

Mr. W. A. WOODARD read, in abstract, a communication dealing with recent developments in the pharmacy of penicillin, streptomycin and dihydrostreptomycin, chloramphenicol, aureomycin and terramycin (*see Review Article, pages* 1009 to 1036).

Mr. W. TRILLWOOD, dealing with the subject from the point of hospital practice, said:—Although a great deal of knowledge is available to the physician, surgeon and pharmacist by the time a new antibiotic is issued for use in hospital, many new problems confront the hospital pharmacist as clinical investigation proceeds and new uses are suggested. Whatever the problem, the pharmacist augments his efforts with the accumulated experience of all whose skill is at the disposal of the hospital service.

Penicillin should be kept cool, dry and sterile—the condition in which it is supplied by the manufacturer. To ensure maximum potency at the time of injection it should be dispensed as it is received in its original container as a dry sterile powder, and the procedure is to supply with the dry powder a sterile solvent and sterile injection apparatus. The pharmacist should provide a sterile syringe service, so that each injection is given with a clean, dry, sterile syringe and a clean, dry, sharp, sterile needle. For intrathecal injections a solution containing a precise concentration of the antibiotic is required, and this must be prepared by the pharmacist. Storage at a low temperature must be ensured by careful labelling, by the regular inspection of ward stocks, and by the withdrawal of out-dated material.

The increasing emergence of penicillin-resistant bacteria had occasioned much discussion. In Oxford, the dosage technique had gone the full circle. With very few minor exceptions no delay-penicillin is used. Instead, penicillin B.P. in high dosage (500,000 I.U.) is given in acute infections, at twelve-hourly intervals; thus providing a germicidal concentration to a wider range of strains of bacteria than procaine penicillin, intermittently it is true; yet it is believed that intermittent bactericidal levels of penicillin are likely to be more effective than continuous bacteriostatic levels.

In view of the increased emergence of insensitive organisms, and the widening bacterial range of antibiotics, sensitivity tests had assumed an

added importance. In requesting sensitivity tests, the physician wants to know what organisms are present, what is the antibiotic of choice, and what is the degree of sensitivity. Bacteriological skill beyond the scope of the pharmacist was required to name and type bacteria; the degree of sensitivity might then be determined by a technician. Sensitivity to penicillin is usually expressed as the concentration of penicillin, in I.U./ml., required to inhibit the organism tested. The question is often posed: an organism has a sensitivity of 0·01, 0·1, 1, 10 or 100 I.U. of penicillin, what dosage of penicillin should be given intramuscularly? The properties of penicillin provide the answer: penicillin is rapidly absorbed and rapidly excreted, blood levels of 10 I.U./ml. are rarely reached. The precise degree of sensitivity is of theoretical interest only; for practical purposes the organisms should be reported simply as "sensitive" for those with a sensitivity below 10 I.U./ml. or "insensitive" where the sensitivity is above 10 I.U./ml.

Bacterial resistance to antibiotics is an ever-present source of anxiety, and many promising new agents have been discarded as organisms have become resistant. It seemed that streptomycin was likely to be short-lived, but, fortunately, the collateral use of *p*-aminosalicylic acid prevents or delays the tubercle bacillus from developing streptomycin-resistance. Unlike penicillin, streptomycin has not been required in diverse pharmaceutical forms. Apart from intramuscular and intrathecal injections and the use of solutions for application to septic granulation areas in plastic surgery, the use of streptomycin is limited. Streptomycin is far more stable than penicillin and does not require any special precautions other than those normally required for the preparation of sterile material. Administration by injection has disadvantages; the injection may be painful, and even when painless, most patients, particularly children, are apprehensive of injections. There has been a general desire for agents which are effective by the oral route, and chloramphenicol, aureomycin and terramycin fulfil this requirement. In addition, these new agents have increased considerably the effective clinical range of antibiotics, notably in providing the first effective weapons for virus and rickettsial infections.

Chloramphenicol is now in general use and is freely available. The use of aureomycin and terramycin is restricted to hospitals, except for certain specified clinical conditions for which general practitioners may obtain supplies through the regional distribution centres.

The shift from antibiotics given parenterally to those effective by mouth is not an unmixed blessing. The rapid absorption, circulation and excretion of penicillin has not resulted in the elimination of the normal bacterial flora of the intestinal tract. This has happened, however, with the oral antibiotics. Not only does the sterilisation of the gut necessitate supplementary administration of vitamin B complex, but a still more disturbing condition may arise. With the sterilisation of both pathogenic and benign organisms, the field seems to be clear for fungal infections, and already deaths have been reported from yeast and monilia infections following the use of oral antibiotics. As the oral antibiotics become more widely used, the demand for fungicidal drugs will increase.

He concluded with an acknowledgment to Dr. R. L. Vollum, Director of the Public Health Laboratories, Oxford, for permission to publish his sensitivity table of antibiotics.

ANTIBIOTICS
Table of Sensitivities

Organism	Penicillin	Streptomycin	Aureomycin	Chloramphenicol	Terramycin
β-*hæmolytic streptococcus*	++++ 0·001 to 0·2*	+++ 0·5 to 120*	+++ 0·3 to 2·5*	++ 0·3 to 2·5*	++? 1·0 to 1·5*
α-*hæmolytic streptococcus*	++++ 0·05 to 5	+++ 2·5 to 120	+++ 0·3 to 2·5	?	+? 0·3 to 2·5
Pneumococcus	++++ 0·001 to 0·2	+++	++ 0·1 to 0·3	?	++? 0·02 to 2·5
Staphylococcus aureus	++++ 0·002 to 1000	++ 3·50	++++ 0·2 to 5	+ 1·5	++? 0·5 to 1·5
Neisseria	++++ 0·002 to 0·3	++ 5·40	++	+	+? 0·2 to 2·5
Clostridium welchii	++++ 0·1 to 1	–	?	?	?
Hæmophilus influenzæ	– 0·2 to 10	+++ 1 to 50	+++ 1 to 5	++ 0·6	+? 2·5 to 4·0
Hæmophilus pertussis	–	–	+?	++ 0·2 to 0·3	? 5·0
Brucella	–	–	++++ 0·75	++++	++? 0·3 to 2·0
Bacterium coli	–	+++ 0·3 to 1000	+++ 5·0	+++ 3 to 10	++ 2·0 to 5·0
Salmonella typhi	–	–	+	++–+ 5	?
Shigella	–	–	+++	+++ 1	?
Proteus	–	+ 1 to 1000	+ 2·5 to 100	+ 10 to 100	+? 50
Pseudomonas pyocyanea	–	++ 2 to 1000	– 10 to 50	– 3 to 100	+? 25
Mycobacterium tuberculosis	–	++++ 0·1 to 1	–	–	–
Rickettsia	–	–	+++	+++	++?
Leptospira	+	–	?	?	?
Treponema pallida	+++	?	+	+	+
Administration	Parenteral	Parenteral	Oral	Oral	Oral
Optimum *p*H	5·5 to 7	7·4 to 9	4 to 5	2 to 9	4 to 7
Toxic effects	Dermatitis	Dermatitis Vertigo Deafness B. deficiency	Nausea Diarrhœa Stomatitis B. deficiency	Nausea Diarrhœa Stomatitis B. deficiency	Nausea Diarrhœa Stomatitis B. deficiency

++++ Probably most effective treatment.
+++ Satisfactory.
++ Moderately satisfactory.
+ Possibly effective.
+? Preliminary reports satisfactory.
– No effect.
? Unknown.
* In vitro sensitivity: In units/ml. for penicillin and in μg./ml. for other antibiotics.

Mr. J. O. DAVIDSON, speaking from the point of view of general pharmaceutical practice, said:—Although our connection with antibiotics is limited to penicillin, streptomycin and chloramphenicol, a large number of preparations of these three substances exists. We have, therefore, to be

prepared to meet a demand quite different from that in the specialised field of the hospital service.

Penicillin occurs in several closely related forms, of which the best known and most important is benzylpenicillin, the soluble sodium salt being the form most frequently met with in general practice.

The preparations most frequently required are:—

Aqueous Injection: Initially, penicillin was injected as a sterile, aqueous solution of from 20,000 to 30,000 I.U./ml. at intervals of from 3 to 4 hours over a 5-day period. The concentration was later increased to 200,000 I.U./ml. and, on occasion, 500,000 I.U. in 2 ml. has been administered at from 4 to 12-hourly intervals. *Oily Injection:* To avoid the dosage frequency of the aqueous solution, oily injection of penicillin B.P., at a concentration of 300,000 I.U./ml. was introduced. Although its high viscosity and liability to cause irritation tend to reduce its popularity, it is still favoured by some practitioners. *Procaine Penicillin:* This is a sparingly soluble salt which is suspended in sterile water before use at a concentration of 300,000 I.U./ml. The suspension retains its potency over relatively long periods. *Fortified Procaine Penicillin* contains 300,000 I.U. with the addition of 100,000 I.U. of benzylpenicillin. *Other Suspension Injections:* Many other suspension injections are available for special conditions. Chief among them is a hydriodide ester which produces a relatively high concentration in the lungs. Another preparation reduces the administration frequency to 24- and even 48-hour intervals. *Tablets:* Penicillin tablets, administered orally when injections are undesirable, usually contain from 100,000 to 200,000 I.U. This is wasteful and expensive, as 5 times the injectable dose must usually be given. *Lozenges, Gums:* A mixture of hard and soft white paraffin, after suitable heat treatment and when sweetened with soluble saccharin and flavoured with oil of peppermint, gives a satisfactory chewing material in which to incorporate penicillin. *Eye-drops:* Penicillin eye-drops, normally containing 15,000 I.U. in 110 minims, should be stored in a cool place and used within 7 days. *Insufflation:* An aural insufflation containing 5000 I.U./g. in sterile sulphathiazole is frequently required for use in a Cade's insufflator. *Oily Nasal Drops:* These are also prescribed at a concentration of 5000 I.U./ml., prepared by dissolving the penicillin in a small amount of distilled water, adding 1 per cent. of phenoxetol as a preservative and making up to volume with Eucerin oil. *Creams, Cones, Dressings, Ointment, Pessaries:* Most of these are now available commercially.

Streptomycin is used either as the hydrochloride or the sulphate or as a double salt formed with calcium chloride. Dihydrostreptomycin B.P., which is formed by the reduction of streptomycin, is used as the hydrochloride or as the sulphate. Its properties, antibacterial activity and therapeutic efficiency, are similar to those of streptomycin and it was thought for a time that this modification considerably reduced the toxicity of the drug. These two substances differ chemically and are not interchangeable.

The preparations most frequently required are:—

Aqueous Injection: A sterile aqueous solution of streptomycin-calcium

chloride complex is most frequently required, the usual strength being either 0·5 g. in 2 ml. or 1·0 g. in 5 ml. It is given intramuscularly in the treatment of tuberculosis, either alone or simultaneously with *p*-aminosalicylic acid. *Eye-drops:* 10 mg./ml. of sterile normal saline. *Eye-ointments:* 0·5 per cent. in sterile eye ointment base. *Solution or Powder for Wounds or Ulcers:* 5 per cent. solution, or 5 to 10 per cent. powder in sterile sulphadiazine. *Cream:* 5 per cent. in a base similar to that used for penicillin cream. *Pessaries:* 100 mg. of streptomycin with 50,000 I.U. of penicillin in oil of theobroma. The streptomycin must be finely powdered to avoid the possibility of small crystals gravitating to the points of the pessaries before they set.

Chloramphenicol occurs as whitish crystals having a very bitter taste. It is only slightly soluble in water but dissolves readily in ethanol and in propylene glycol giving stable solutions. It is absorbed well after administration by the mouth. It is given in the following forms:— *Capsules:* Hard capsules each containing 250 mg., the recommended daily doses being 30 mg., 75 mg., or 100 mg./kg. bodyweight in suitably divided doses. *Palmitate Suspension* for infants and children unable to swallow capsules. This non-bitter derivative hydrolyses in the small intestine, liberating chloramphenicol. Two teaspoonfuls are equal to one 250-mg. capsule. *Suppositories* each containing 125 mg. in cocoa butter. *Eye-drops:* either a 0·25 per cent. solution (saturated) or a 0·5 per cent. borate buffered solution of the drug. *Eye-ointment:* 1 per cent. in oculentum base. *Cream:* 1 per cent. in hydrous emulsifying ointment. *Dusting Powders:* 2 to 3 per cent. in dried sterile lactose. *Ear-drops:* 10 per cent. in propylene glycol. *Use in Dentistry:* Recently, a suspension of 1 g. each of chloramphenicol, streptomycin and sodium caprylate with 3 ml. of propylene glycol has been required for insertion into inflammatory root areas of teeth via the root canal. The function of sodium caprylate is to inhibit the action of the yeasts which are unaffected by the antibiotics.

Mr. H. A. TURNER (Nottingham) observed that the subject of antibiotics, which began in the academic field, had now passed into the hands of manufacturers. The B.P. statement that benzylpenicillin was so stable that when heated in an open vial at 100° C. for four days it lost not more than 10 per cent. of its potency spoke well for the quality of the material now available.

Mr. A. F. CALDWELL (Singapore) said that temperatures should be stated more precisely than by the word "normal." This was of importance to workers in tropical countries. With regard to the oral use of antibiotics, in Singapore trouble had been experienced with monilia and fungus infections. There was also the problem of allergic sensitivity, especially in patients using penicillin lozenges.

Mr. A. STERLING (London) said that the length of treatment with antibiotics and the cost were important in hospital practice. No mention had been made of drugs which delayed excretion of the antibiotics. For instance, was there any substance which was not toxic to the kidney and which would delay excretion of penicillin?

Mr. J. M. MYERS (Bradford) asked Mr. Woodard whether there was a

suitable solvent for aureomycin in 2 to 3 per cent. solution for topical application. Was there any evidence that chloramphenicol and aureomycin were inactivated by specific enzymes in the way that penicillin was inactivated by penicillinase? He asked for information regarding staff, cost and type of syringe required for the maintenance of a sterile syringe service for a 500-bed hospital. Was a mixture of chloramphenicol and streptomycin efficacious for sterilising root canals, and was it good practice to continue the use of antibiotics in dentistry, because of sensitisation resulting from continued local application?

Mr. P. CLAPHAM (Speke) said he understood that "tweens" were normally used in preparing aqueous injections, and inquired whether they could be used for oily injections? Four days seemed a short period for the storage of buffered penicillin solutions. Advances were taking place in which higher concentrations of soluble penicillin added to procaine penicillin were made available. There was room for the development of injections which enabled higher doses of soluble penicillin to be given with the usually accepted doses of procaine penicillin. This would achieve a bacteriostatic level of soluble penicillin, and would maintain a suitable blood level over a period, thus reducing the number of injections. Generally, sodium carboxymethylcellulose was used in very small quantities, and if the grade was carefully selected it was a very useful addition. There were improved forms available which produced an almost clear solution.

Mr. J. H. OAKLEY (London), referring to the improved ointment base in the B.P. Addendum 1951, gave figures showing the improvement in stability using potassium benzylpenicillin. A 5 per cent. loss of potency occurred in 12 months when the ointment was stored at 5° C., at 20° to 28° C., or at 35° C. Lozenges were now so stable that there was no significant loss in twelve months at the above temperatures, provided they were kept dry. With solution-tablets there was a 10 per cent. loss of potency in 12 months. Here, most of the losses occurred just after manufacture, but if the tablets were buffered there was no loss of potency after 8 months. It was a pity that the eye ointment of penicillin was not changed at the same time as the ointment.

Mr. T. D. WHITTET (London) said that since the introduction of penicillin in lactose for topical application the use of sulphonamides as diluents in powders had, in general, been abandoned at his hospital. Streptomycin with maphenide had been used topically, and sulphacetamide with penicillin powder had been useful for infected root canals. In making potassium benzylpenicillin with lactose powder a loss of potency of 40 to 50 per cent. was obtained using dry heat at 150° C. even when the lactose had been previously dried. Chloramphenicol could be sterilised by dry heat at 150° C. for one hour. An intrathecal injection of chloramphenicol containing 2·5 mg./ml. could be sterilised by autoclaving.

Mr. F. H. OLIVER (Sunderland) said that the determination of the sensitivity of the organism to the antibiotic would lead to a saving in the antibiotics and would prevent their misuse. In his view the determination of the sensitivity of organisms was an excellent example of pharmacy as

opposed to dispensing, to which the pharmacist could make a valuable contribution.

Mr. J. C. HANBURY (Ware) said it was clear that the dangers inherent in the use of the newer antibiotics were so serious that the continued use of penicillin, with its lower toxicity, was called for. In his view more discrimination was needed in the use of ointments, creams and lozenges of penicillin in local infections. The pharmacist should familiarise himself with the use and abuse of antibiotics.

Mr. R. L. STEPHENS (Brighton) suggested that the stability of penicillin oral tablets and lozenges would be improved by replacing the cottonwool packings with plastic or nylon. Nylon yarn had a water absorption of under 1 per cent., whereas cotton wool normally absorbed about 8 per cent. of moisture, which must adversely affect the storage life of the tablets. At the same time the use of a coloured silica gel moisture indicator might be of advantage. Had Mr. Woodard any experience of the combination of two or more antibiotics in one preparation or of their simultaneous use? In particular, the combination of streptomycin and penicillin was very popular with ships' doctors, and he asked why more of it had not been seen in this country.

Mr. E. MATTHEWS (Portsmouth) said that in cases of shock it was necessary, in order to get absorption, either to use intra-arterial injection or to add a spreading agent, such as hyaluronidase, and he asked for information on the formulation of such injections. Was anything known of the formulation of antibiotics with an antihistamine, and would it be pharmacologically sensible as a means of preventing anaphylactic emergencies?

Mr. D. M. BRYCE (Barnet) asked Mr. Woodard what was his experience of sodium carboxymethylcellulose, and whether he was aware of any tissue damage following injection.

Mr. W. P. LEGGETT (Speke) referred to the co-operative work on bacteriostatics which was going on in the laboratories of penicillin manufacturers and others interested. The papers suggested a return to the use of soluble penicillin salts and it might be that developments towards improvements in buffering would follow. Most of the bacteriostatics tried had failed owing to incompatibility. They had turned, therefore, to the newer agents, such as the esters of *p*-hydroxybenzoic acid and the quaternary ammonium compounds. These were compatible, and the former were quite effective against *Pseudomonas pyocyanea*, though they did not suppress certain resistant strains of staphylococci: the latter, on the other hand, "almost fertilised" *Ps. pyocyanea*.

Mr. J. R. ELLIOT (London) said that at his hospital it had been found possible to reduce the concentration of aureomycin in ointments from 3 to 1 per cent. and obtain similar clinical results. Why did Mr. Trillwood prefer penicillin as a dry powder to a ready-made injection issued from the pharmacy? Multiple dose containers holding 5 to 10 mega-units of penicillin had been found useful so that supplies would be used up within the stated pharmacopœial life. Injections in multiple dose containers were cheaper and easier to prepare.

Mr. A. MARSH (Brighton) stated that sensitivity tests were part of the duties of the pharmacist, because not every hospital had the services of a bacteriologist or laboratory technician. He agreed with Mr. Hanbury that the pharmacist should know all the properties of the antibiotics, including how to test for sensitivity.

Mr. R. W. GILLHAM (Leeds) referred to some unusual preparations, including a mixture of penicillin with blood plasma, penicillin tampons, penicillin with talc, and dihydrostreptomycin as a bulk injection of 50 ml. containing 1 g./ml. with a dose of 0·5 ml. for a child; this gave a solution of treacly consistency.

Dr. E. I. SHORT (Beckenham) said sensitivity tests acted as a control on excessive prescribing of antibiotics. Had Mr. Trillwood any experimental or clinical evidence to support his belief that intermittent bactericidal levels of penicillin were more effective than continuous bacteriostatic levels?

Mr. H. WILLIAMS (Reading) said that in his experience general practitioners were tending to prescribe mixtures containing penicillin in preference to injections. Could Mr. Davidson suggest the best solvent and say how he would dispense such a preparation?

Mr. N. A. HERDMAN (Speke) said that leading dermatologists were not in favour of topical penicillin preparations. The variation in the views of doctors on such treatment led him to suggest that the collection and correlation of medical information might be the subject of a future symposium.

Mr. S. POWLSON (London) queried the statement that chloramphenicol could be sterilised at 150° C. The suppliers of the drug suggested that a suitable method of sterilisation was to heat the chloramphenicol in fine powder at 105° C. for one hour. That had been issued as a suspension in water at a strength of 1 or 2 g. in 10 ml. for injection into the pleural cavity. Aureomycin was being used in concentrated solution as an ærosol.

Mr. C. W. ROBINSON (Liverpool) said that a number of different brands of penicillin lozenges described as "B.P." contained added colour and flavour. It would be of advantage for the Pharmacopœia to lay down standards.

Miss I. HARRIS (Bromley) pointed out that penicillin and chloramphenicol were frequently prescribed to be administered together and asked whether the simultaneous administration of penicillin and other antibiotics was advantageous.

Mr. D. F. SMITH (Bournemouth) said that he had used with success polyvinylpyrrolidine in preparing a chloramphenicol suspension for introduction into the pleural cavity. With regard to therapy with two or more antibiotics, American workers had found that while penicillin and streptomycin could be administered concurrently and with complementary effects, penicillin or streptomycin was antagonistic to other antibiotics, such as chloramphenicol or aureomycin.

Mr. H. TREVES BROWN said that Mr. Trillwood referred to the use of vitamin B complex tablets to mitigate the effects of antibiotics when administered orally. It would be interesting to know whether the view

was still held that they had some effect. Their use started when the occurrence of black tongue was reported and somebody found that nicotinamide and nicotinic acid cured it. Then it was suggested that as the condition occurred suddenly, it could not be a vitamin deficiency, and he understood that the prevailing view was that the vitamin B complex was not a cure for the symptoms following oral treatment with antibiotics.

Mr. F. G. WELLS (London) said he wondered if it was realised how seldom the retail pharmacist had an opportunity to use his knowledge of antibiotics, and how the medical man would receive any attempts to tell him how to prescribe? The main worry in retail work was the present multiplicity of preparations on the market. The use of proprietary names necessitated the stocking of more than one brand of an antibiotic preparation, and the matter was not being viewed from the pharmaceutical, but rather from the commercial, point of view.

Mr. J. JACOBS (Sunderland) referred to tyrothricin and bacitracin, which were used topically. More information on these was desirable.

Mr. J. O. DAVIDSON, in reply, said that in the use of suspensions in dentistry, the aim was to stop the formation of chronic ulcers in the root area. Mr. Clapham's remarks concerning procaine penicillin and his defence of it as an injection were interesting. The fact that the oily injection of penicillin was going out of use was rather surprising, because it seemed much more logical to inject a little oil rather than procaine Lozenges were being used very widely for all purposes, but the sterile cream was falling out of favour. It was not his experience that the injection of penicillin was giving place to oral administration in mixtures. In his opinion an aqueous solvent was the best for penicillin.

Mr. W. A. WOODARD, in reply, agreed that it was important to specify temperature. In preparing oily suspensions "tweens" and similar preparations had been used with partial success. Aluminium stearate gave a permanently stable suspension when the preparation was properly formulated and prepared. Plastic materials were coming into use for the protection of tablets in place of cotton wool. Combinations of steptomycin and penicillin were extensively used in the United States. Because of the wider spectrum effect such combinations were active against Gram-negative and Gram-positive organisms, and were clinically more effective. Incorporation of soluble forms of penicillin reduced their stability in procaine penicillin preparations. Much depended on the degree of substitution in sodium carboxymethylcellulose. It was reasonably safe material, provided it was used in small amounts, and the correct grade chosen. The only way of determining whether a grade was suitable was by animal tests and clinical trials. Agreeing with Mr. Leggett, bacteriostatics in the suspension type of product could frequently give rise to more trouble than in penicillin solutions. Bacteriostatics were often not compatible with added suspending and wetting agents. He did not consider that the B.P. went far enough in the control of penicillin lozenges and other preparations of that type.

Mr. W. TRILLWOOD, in reply, said that he had no experimental evidence to offer with regard to the desirability of having intermittent bactericidal

levels of penicillin rather than continuous bacteriostatic levels. However, work was being done at Oxford because there was evidence that in a number of hospitals something like 30 to 35 per cent. of all septic cases had resistant organisms. It was felt that that had largely been caused by under-dosage with penicillin. It was also thought that it might have been caused by the use of delayed action penicillin giving a long continuous bacteriostatic level. Penicillin in the right dosage was a bactericide and should be used as such. It was impossible for the general practitioner, and difficult for the nursing staff, to administer injections three-hourly in order to maintain a continuous bactericidal level; but there was a school of thought which took the view that it was a good thing to let penicillin completely disappear from the blood for a period. The best time to strike bacteria was when they were in an active state of metabolism. With regard to the cost of treatment with antibiotics, 30 drugs accounted for two-thirds of the hospital drug bill and the five substances: penicillin, streptomycin, chloramphenicol, aureomycin and *p*-aminosalicylic acid accounted for 50 per cent. A sterile syringe service would always be expensive. A saving could be made in the avoidance of breakages by the use of skilled technicians In his own case the breakage rate was less than $\frac{1}{2}$ per cent. The case for sensitivity tests had been stated in the paper. If a mixed culture were being investigated the doctor would want to know what organisms were present, and what was the antibiotic of choice. In his experience local applications of penicillin or antibiotics were not used to any great extent. Streptomycin was used in plastic surgery. He believed in keeping penicillin powder dry because a sterile syringe service was provided which was essential quite apart from antibiotics. Hospital pharmacists knew that ward stock should be regularly turned over to ensure that solutions of penicillin would not be inactive when administered to the patient but could they always be sure that this was done? With the dry powder, and by allowing the nursing staff to add sterile water with sterile apparatus, as much as possible had been done to ensure that the patient received a penicillin injection of the strength which the prescriber ordered. He was unable to answer the point raised by Mr. Treves Brown.

RESEARCH PAPERS

THE EFFECT OF PENICILLIN AND OF STREPTOMYCIN ON BLOOD COAGULATION IN NORMAL SUBJECTS

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Introduction

A number of workers have presented laboratory evidence that penicillin and streptomycin affect the clotting of blood, and it has been suggested that recumbency thrombosis might be precipitated by their use. A study of the literature shows a conflict of experimental evidence, and it appears that much of the reported work has been imperfectly controlled. It was therefore thought worth while to reinvestigate this problem in healthy human volunteers by using an experimental design in which the effects of person-to-person and day-to-day differences would not bias the results, and to study a number of clotting tests.

Review of the Literature

The experimental literature is summarised in Table I. Impressions of corresponding clinical effects have been equally varied. Frada,[1] Courty and Biscaye[2] and Ochsner *et al.*[3] suggested that penicillin treatment might contribute to the occurrence of thrombosis in ill persons, while, on the

TABLE I

Summary of the literature

These reports deal with effects in man and in laboratory mammals: the tabulation does not refer to the *in vitro* experiments which some of the authors also describe

Drug	Effect reported		
	Coagulant	Anticoagulant	None
Penicillin	Moldavsky *et al.*[32] Macht[20,21,22]	Hines and Kessler[4] (potentiation of heparin)	Ungar[38] Lewis[34] (including hæmophilia) Macht and Ostro[23] (hæmophilia) Weiner *et al.*[35] Dolkart *et al.*[36] (including tests with heparin) Reggianini[37]
Streptomycin	Macht (*ibid.*) Donatelli and Pasquinucci[38] Meneghini *et al.*[39]	Giannico and Provini[40]	Farrington *et al.*[41] Nassi and Ulivelli[42] de Michele and Portella[43] Elson[44]

* In receipt of a grant from the Medical Research Council.

other hand, Hines and Kessler[4] thought that penicillin might contribute to the occurrence of hæmorrhage in cases of subacute bacterial endocarditis under combined treatment with penicillin and heparin.

EXPERIMENTAL

3 experiments were made, the drugs being given parenterally. The first 2, each with 6 subjects, investigated penicillin and streptomycin separately and were similar in design. The third, with 2 sets of 4 subjects, included both drugs.

1. *Experiments* 1 *and* 2

Drug Administration

Experiment 1: *Penicillin.* Tests were made about 15 minutes after a single injection of 0·5 mega-units of crystalline sodium penicillin, and again 12 to 24 hours after the last of 4 daily injections of 0·6 mega-units of crystalline procaine penicillin G.

Experiment 2: *Streptomycin.* Tests were made about 15 minutes after a single injection of 1·0 g. of dihydrostreptomycin sulphate, and again 12 to 24 hours after the last of 3 daily injections of the same quantity given on the days following the first test.

The first series of tests with each drug was designated "immediate," and the second series "delayed."

TABLE II

PAIRING OF DRUG AND CONTROL SUBJECTS IN EXPERIMENTS 1 AND 2

The same design was used with different subjects in the two experiments

Week and day of test	Test	Drug subject	Control subject
Week 1. Monday Week 1. Friday	Immediate Delayed	I	IV
Week 2. Monday Week 2. Friday	Immediate Delayed	II	V
Week 3. Monday Week 3. Friday	Immediate Delayed	III	VI
Week 4. Monday Week 4. Friday	Immediate Delayed	IV	
Week 5. Monday Week 5. Friday	Immediate Delayed	V	II
Week 6. Monday Week 6. Friday	Immediate Delayed	VI	III

Controls. It was not practicable to test all 6 subjects on each experimental day, so they were tested in pairs according to the scheme of Table II. In both experiments the six subjects thus each provided a "drug" and a "control" reading in the immediate and in the delayed tests, with an interval between the drug and control readings on any one subject. The paired tests were made strictly in parallel.

In these experiments the control subjects did not receive inert injections.

Tests Used

(i) *Clotting tests. The whole blood clotting time* was measured on venous blood by the method of Lee and White[5] at 37° C. in ordinary glass

tubes 6·5 × 0·9 cm. and in similar tubes silicone-coated (in both cases taking the mean clotting time from 4 tubes at each venepuncture), and on capillary blood by the bead-capillary method of Dale and Laidlaw,[6] taking the mean of single readings obtained from a stab wound in each ear lobe.

The "prothrombin consumption" was measured 1 hour after venepuncture on the "glass" and "silicone" sera from the Lee-White tubes, by observing

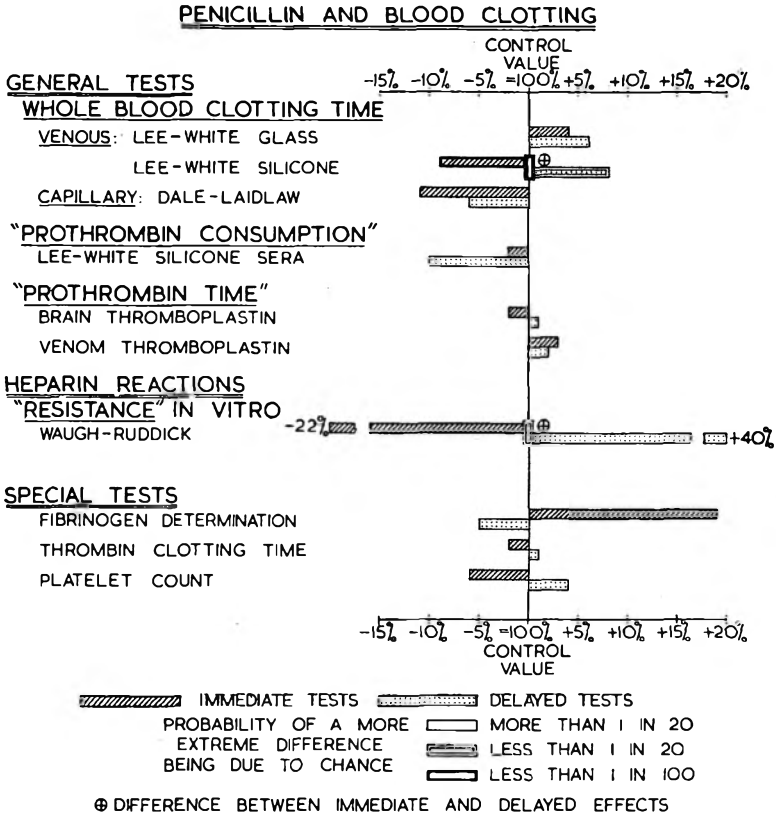

FIG. 1. *Experiment* 1: *Penicillin.* Magnitudes of differences between test and control results for each test with indications of statistical significance. The penicillin values are shown as the percentages by which they differ from the respective control values, which are taken as 100 per cent. For the de Takats test, see Fig. 2.

the clotting times of mixtures of 0·1 ml. of serum, fibrinogen solution, M/40 calcium chloride solution and acetone-dried human brain extract (Biggs and Macfarlane[7]). The mean was taken of 2 replicate readings in Experiment 1 and of 4 in Experiment 2. None of the "glass" sera clotting times was pathologically short. All the "silicone" sera clotting times were therefore accepted for analysis.

The plasma "prothrombin time" was measured by the one-stage technique with both human brain thromboplastin as above and with Russell's

viper venom, taking the mean of 2 to 4 replicate readings on each plasma sample.

The heparin "resistance" test (Waugh and Ruddick[8,9]) was made on citrated whole blood against 6 concentrations of heparin, a single series being tested with each sample. The results were summarised as the slope of the regression of clotting time on heparin concentration.

The heparin "tolerance" test (de Takats[10]) was made by giving an intravenous injection of heparin after the venepuncture and the Dale-Laidlaw tests; in Experiment 1, 500 U./stone of body-weight were given, and in Experiment 2, 5000 U. to each subject. Thereafter at intervals

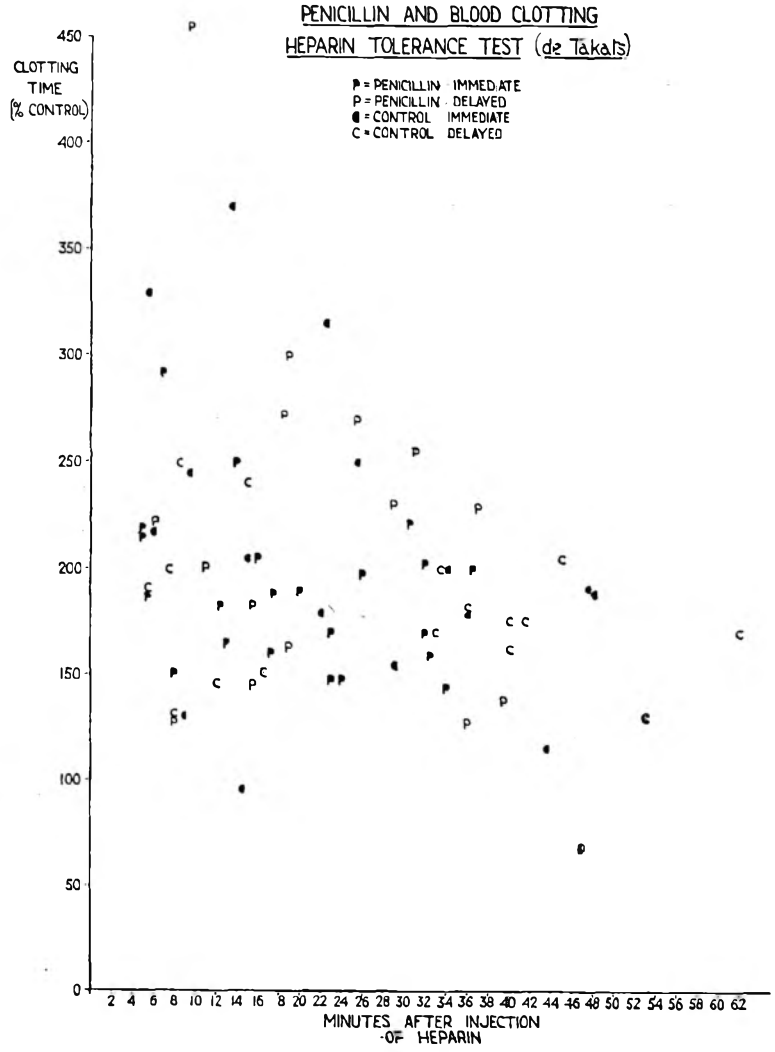

FIG. 2. *Experiment* 1: *Penicillin.* Observed results in the de Takats test (heparin "tolerance," *in vivo*) in control and penicillin subjects.

the Dale-Laidlaw clotting times were obtained from alternate ear lobes and were expressed as percentages of the mean Dale-Laidlaw clotting time before the injection of heparin. This test was abandoned in Experiment 2 because of the magnitude of the experimental error.

The plasma fibrinogen concentration was determined by a clot-weight method (Ingram[11]) on a single plasma sample at each testing.

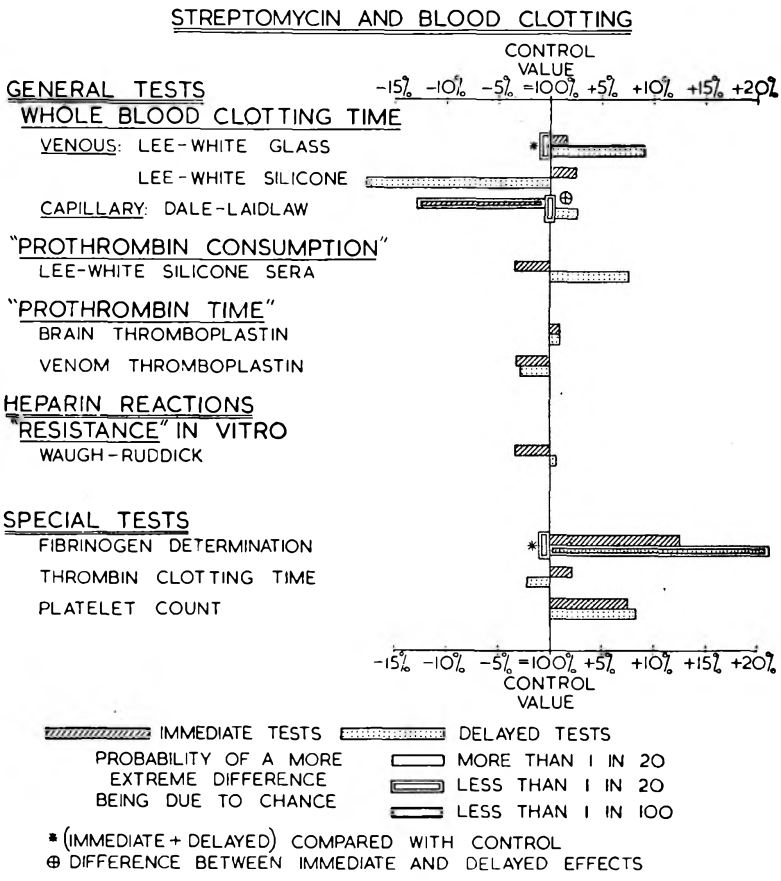

FIG. 3. *Experiment* 2: *Streptomycin.* Magnitudes of differences between test and control results for each test with indications of statistical significance. The streptomycin values are shown as the percentages by which they differ from the respective control values, which are taken as 100 per cent. For the de Takats test, see Fig. 4.

The thrombin clotting time was measured on citrated plasma with solutions of purified human thrombin (Lister Institute), the mean of 2 to 4 replicate tests being taken.

Platelet counts were made on capillary blood by the method of Baar.[12]

(ii) *Other tests. Serum drug concentrations* were estimated by the tube dilution method using the Oxford staphylococcus for penicillin and the Klebsiella K41 for streptomycin.

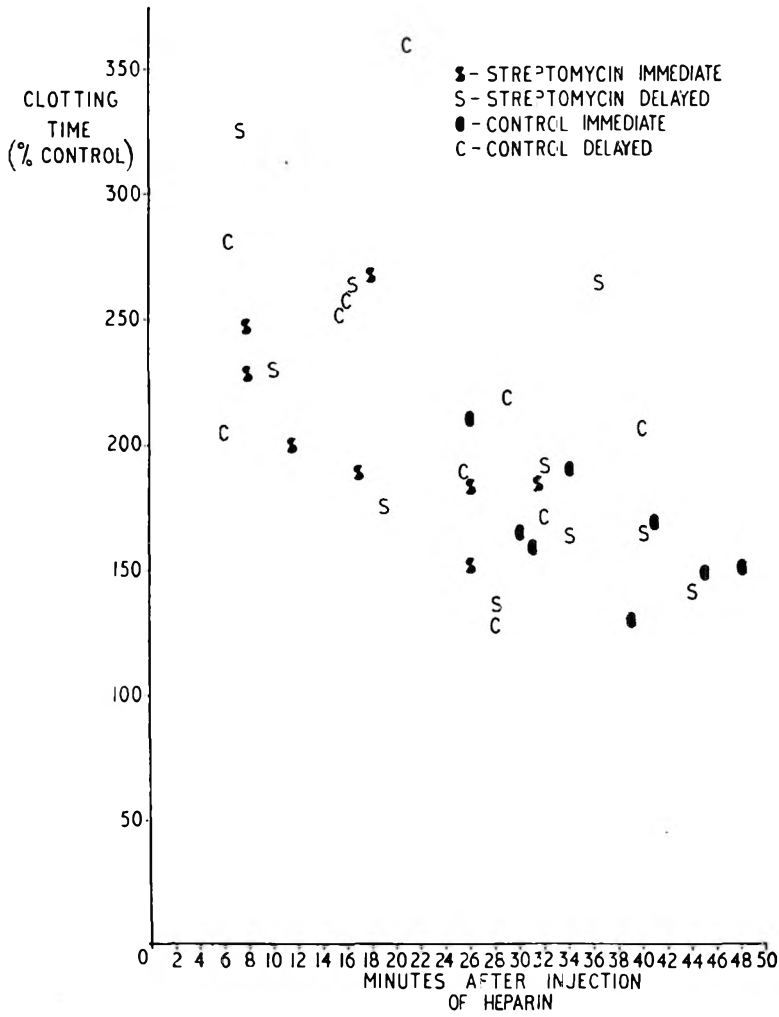

FIG. 4. *Experiment* 2: *Streptomycin.* Observed results in the de Takats test (heparin "tolerance," *in vivo*) in control and streptomycin subjects.

Plasma fats were estimated as it was thought that the random variation in the clotting tests might be reduced by taking into account the plasma concentrations of fatty substances (Waldron and Friedman[13]). The following techniques were used: *total fatty acids* by the method of Stewart and Hendry[14]; *total cholesterol* by the method of Sackett[15]; *free cholesterol* by the method of Schoenheimer and Sperry[16]; and the *lipoid phosphorus* by the method of Stewart and Hendry.[17]

2. *Experiment* 3

Drug Administration and Controls

2 groups each of 4 subjects were submitted to immediate tests only, testing all 4 subjects on each experimental day according to the scheme of Table III, and testing the 2 groups on different days. The subjects received on different days one of the following injections: 1·0 mega-unit of

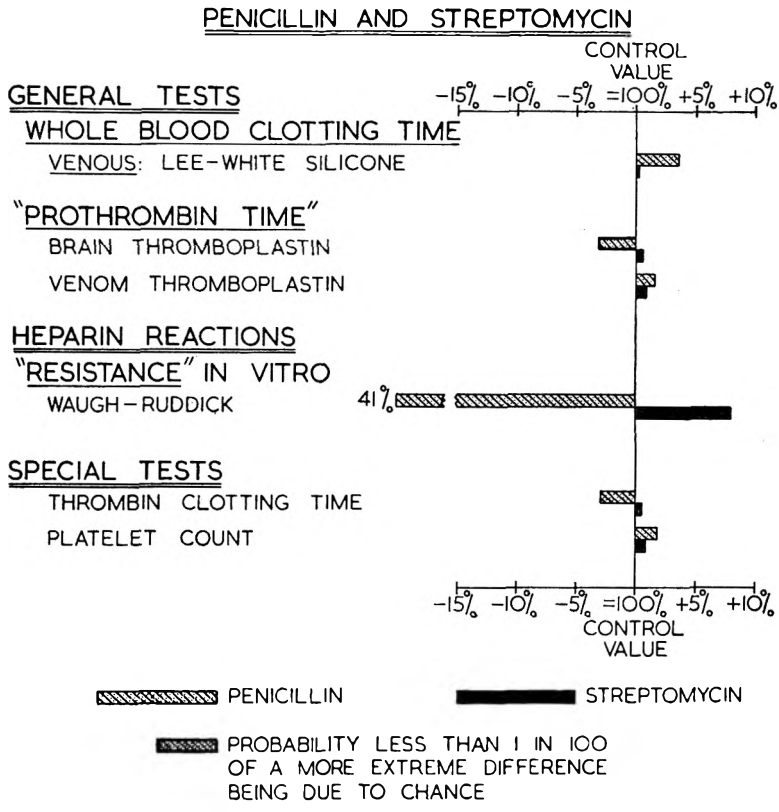

FIG. 5. *Experiment* 3: *Penicillin and streptomycin.* Magnitudes of differences between test and control results for each test (immediate effects only). The test results are shown as the percentages by which they differ from the respective control results, which are taken as 100 per cent.

crystalline sodium penicillin G; 2·0 g. of dihydrostreptomycin sulphate; 0·3 mg. of adrenaline tartrate (synthetic); and saline solution. The adrenaline and the saline solution were given as controls of emotional responses to the investigation: the subjects believed in fact that 4 different batches of penicillin were under investigation. Since they were not aware that they might receive adrenaline, and it was, thus, not desirable to make any systematic clinical observations for signs of adrenaline activity in the subjects concerned, indices of adrenaline activity were sought by platelet counting and by making blood sugar estimations.

TABLE III

GRAECO-LATIN ARRANGEMENT USED IN ALLOCATING INJECTIONS AND ORDER OF INJECTIONS IN EXPERIMENT 3

P = penicillin; S = streptomycin; a = adrenaline; s = saline
Suffix numerals determine the order of injecting the subjects on each day. The order of injection departs from the balanced arrangement required for a Graeco-Latin square but the other, more important, factors are perfectly balanced.

Group (i)					Group (ii)				
Week and day of test	Subjects				Week and day of test	Subjects			
	I	II	III	IV		V	VI	VII	VIII
Week 1. Monday	s_4	P_3	S_1	a_2	Week 1. Friday	s_4	P_3	S_1	a_2
Week 2. Monday	S_3	a_4	s_2	P_1	Week 2. Friday	S_3	a_4	s_2	P_1
Week 3. Friday	a_1	S_4	P_3	s_2	Week 3. Monday	a_1	S_4	P_3	s_2
Week 4. Friday	P_2	s_1	a_3	S_2	Week 4. Monday	P_2	s_1	a_3	S_4

Tests Used

(i) *Clotting tests.* The Lee-White silicone *whole blood clotting time* as above.

The plasma "prothrombin time" as above.

The heparin "resistance" test was made on slow-spun citrated plasma, following the modification of Silvermann[18] and testing 4 heparin concentrations. The results were summarised as before.

The thrombin clotting time as above.

The platelet count on venous blood, otherwise as above.

In the plasma prothrombin time and thrombin clotting time tests the samples from the 4 subjects were tested concurrently, 4 replicates being obtained from the venepuncture on each subject on each day. The 4 samples were also examined concurrently in the heparin "resistance" test, 1 reading being obtained from the venepuncture on each subject against each heparin concentration on each day. In all these tests, randomised Latin square arrangements were used to eliminate systematic errors on order of testing.

(ii) *Other tests. Serum concentrations of penicillin and streptomycin* were determined as above.

The blood sugar was determined on oxalated blood by the method of Hagedorn and Jensen.[19]

RESULTS

Presentation of Results

Experiments 1 and 2 each provided about 1000 individual readings and Experiment 3 about 400. Raw data are therefore not usually given but the summarised data are arranged principally to indicate the magnitude and significance of the observed differences between mean drug and control results. The standard errors of the differences were obtained from analyses of variance in which overall differences between subjects and between days have been eliminated from the treatment comparisons.

1. *Serum concentrations of penicillin and streptomycin.* Table IV shows the serum concentrations of penicillin and of streptomycin obtained in the 3 experiments. The accuracy of the determinations is subject to the usual limitations of twofold dilution assays.

2. *Adrenaline activity.* Table V shows the mean platelet counts and the mean blood sugar concentrations observed in the adrenaline and saline subjects in Experiment 3. It appears that in neither test was the dose of adrenaline sufficient to evoke a response. As significant differ-

TABLE IV

SERUM CONCENTRATIONS OF PENICILLIN AND OF STREPTOMYCIN OBTAINED IN THE DRUG SUBJECTS IN THE THREE EXPERIMENTS

Except at the extreme concentrations, the results are expressed as the mean of the tested concentrations just permitting and just inhibiting the growth of the test organism. The entries are derived from the 6, 6 and 8 subjects respectively

Experiment 1 Penicillin I.U./ml.		Experiment 2 Streptomycin I.U./ml.		Experiment 3	
Immediate	Delayed	Immediate	Delayed	Penicillin (immediate) I.U./ml.	Streptomycin (immediate) I.U./ml.
7·5	0·03	80	10	20	20
15	0·06	40	10	15	40
15	0·03	20	2·5	30	40
4	0·03	5	2·5	15	20
15	0·03	20	10	15	10
7·5	0·1	40	2·5	0·06*	1·2*
				30	20
				20	15

* These low values occurred in different subjects on the same day. In both cases a quantity of the drug was lost at the time of injection.

TABLE V

PLATELET COUNTS AND BLOOD SUGAR CONCENTRATIONS OBSERVED IN ADRENALINE AND SALINE SUBJECTS IN EXPERIMENT 3

8 observations were available in each case

S.E. = Standard error of the difference between the means
P = Probability of obtaining, by chance, a difference more extreme than that observed

Test	Adrenaline subjects		Saline subjects		Difference ± S.E. between means, with significance
	Mean	(Range)	Mean	(Range)	
Platelet count thousands/cu. mm.	320	(261–390)	331	(262–390)	-11 ± 15 $(0{\cdot}4 < P < 0{\cdot}5)$
Blood sugar mg. per cent. ..	96·8	(87–112)	94·5	(69–147)	$2{\cdot}3 \pm 4{\cdot}8$ $(0{\cdot}6 < P < 0{\cdot}7)$

ences are not apparent in the clotting data from these two groups, this material has therefore been pooled in the subsequent analysis, with the exception of the "prothrombin time" test with brain thromboplastin as shown in Table VIIIB.

3. *Effects due to penicillin and to streptomycin.* The analyses of the results of the 3 experiments are given in Tables VI-VIIIA and B. A number of the drug-control differences are shown as statistically significant. The significance is probably spurious in the immediate Lee-White silicone test of Experiment 1 and in the delayed Lee-White glass test of Experiment 2, because in these cases the estimate of residual variation used in the analysis of variance is, by chance, actually smaller than its error component.

(The estimates of the total residual variances, derived from the analyses of variance, are based on at most 7 degrees of freedom in Experiments 1 and 2, and 12 in Experiment 3. For each test a separate estimate of the

experimental error, which is one component of the total variance, can be obtained from the scatter between replicates, and, being based on a large number of degrees of freedom, is relatively more accurate. These error components were therefore always estimated as a check on the residual variances, whenever replicate readings were obtained.)

The magnitudes of drug-control differences are shown diagrammatically in Figures 1, 3 and 5, except for the de Takats tests, of which the actual data are shown as scatter diagrams in Figures 2 and 4. Treatment effects

TABLE VI

EXPERIMENT 1. THE EFFECTS OF PENICILLIN

Test	Immediate observations			Delayed observations		
	Drug subjects' mean	Control subjects' mean	Difference in means ± S.E.	Drug subjects' mean	Control subjects' mean	Difference in means ± S.E.
1. Lee-White (glass) (minutes) ..	13·08	12·58	+0·50 ± 0·72	12·78	12·02	+0·76 ± 0·72
2.‡Lee-White (silicone (minutes) ..	39·13	43·13	−4·00** ± 0·88	39·05	36·32	+2·73* ± 0·88
3. Dale-Laidlaw (minutes) ..	2·14	2·41	−0·27 ± 0·18	2·19	2·34	−0·15 ± 0·18
4. "Prothrombin consumption" (seconds) ..	21·5	22·0	−0·5 ± 1·6	21·8	23·9	−2·1 ± 1·6
5. "Prothrombin time" (brain thromboplastin) (seconds) ..	15·30	15·65	−0·35 ± 0·22	16·01	15·85	+0·16 ± 0·22
6. "Prothrombin time" (venom thromboplastin) (seconds) ..	15·17	14·68	+0·49 ± 0·37	13·80	13·50	+0·30 ± 0·37
7. Thrombin clotting time (seconds) ..	7·66	7·82	−0·16 ± 0·13	7·00	6·93	+0·07 ± 0·13
8.†Waugh-Ruddick (heparin) (minutes/u. heparin)‖ ..	34·0	43·6	−9·6 ± 7·1	50·9	36·5	+14·4 ± 7·1
9. Platelet count (thousands/cu. mm.)	316	335	−19 ± 30·0	399	384	+15 ± 30
10. Fibrinogen concentration (g. per cent.) ..	0·305	0·256	+0·049 ± 0·028	0·254	0·266	−0·012 ± 0·028

* Different significant at the 5 per cent. level.
** Different significant at the 1 per cent. level.
† Difference between immediate and delayed drug values significant at the 5 per cent. level.
‡ Difference between immediate and delayed drug values significant at the 1 per cent. level.
‖ Results are expressed as the slope of the regression of clotting time on heparin concentration.

S.E. = Standard Error of the difference between the means.

Note that for an analysis with no missing readings there are only 7 degrees of freedom for error: where $n = 7$, $t = 2{\cdot}365$ for $P = 0{\cdot}05$.

in the Lee-White whole blood clotting times were also non-significant when studied as the ratio of the silicone time to the glass time.

4. *Correlation between clotting times and blood fats* (*Experiments* 1 *and* 2). It was thought that if a significant correlation could be demonstrated between clotting times and blood fats the effect would be most obvious in the Lee-White silicone whole blood clotting time and in the venom "prothrombin time." In neither case was a significant correlation found and thus no reduction in random error could be obtained. All the fat values were thought to be within normal limits for the several determinations.

TABLE VII

EXPERIMENT 2. THE EFFECTS OF STREPTOMYCIN

Test	Immediate observations			Delayed observations		
	Drug subjects' mean	Control subjects' mean	Difference in means ± S.E.	Drug subjects' mean	Control subjects' mean	Difference in means ± S.E.
1. Lee-White (glass) (minutes) ..	13·02	12·82	+0·20 ± 0·30	13·23	12·11	+1·12** ± 0·30
2. Lee-White (silicone) (minutes) ..	32·10	31·32	+0·78 ± 5·05	28·78	34·98	−6·20 ± 5·05
3.†Dale-Laidlaw (minutes) ..	1·66	1·90	−0·24* ± 0·08	1·77	1·73	+0·04 ± 0·08
4. "Prothrombin consumption" (seconds) ..	21·8	22·6	−0·8 ± 3·2	18·4	17·1	+1·3 ± 3·2
5. "Prothrombin time" (brain thromboplastin) (seconds) ..	14·12	13·98	+0·14 ± 0·19	13·98	13·85	+0·13 ± 0·19
6. "Prothrombin time" (venom thromboplastin) (seconds) ..	14·17	14·63	−0·46 ± 0·73	14·99	15·44	−0·45 ± 0·73
7. Thrombin clotting time (seconds) ..	10·68	10·47	+0·21 ± 0·16	9·40	9·60	−0·20 ± 0·16
8.‖Waugh-Ruddick (heparin) (minutes/u. heparin) ..	24·5	25·4	−0·9 ± 5·8	18·7	18·6	+0·1 ± 5·8
9. Platelet count (thousands/cu. mm.)	323	305	+23 ± 24·0	288	266	+22 ± 24
10. Fibrinogen concentration (g. per cent.) ..	0·340	0·308	+0·032 ± 0·017	0·394	0·325	+0·069* ± 0·017

* Difference significant at the 5 per cent. level.
** Difference significant at the 1 per cent. level.
† Difference between immediate and delayed drug values significant at the 5 per cent. level.
‖ Results are expressed as the slope of the regression of clotting time on heparin concentration.

S.E. = Standard Error of the difference between the means.

Note that for an analysis with no missing readings there are only seven degrees of freedom for error: where $n = 7$, $t = 2{\cdot}365$ for $P = 0{\cdot}05$.

TABLE VIIIA

EXPERIMENT 3. THE EFFECTS OF PENICILLIN AND OF STREPTOMYCIN

Test	Pooled means for adrenaline and saline tests (= control)	Penicillin tests (immediate)		Streptomycin tests (immediate)	
		Mean	Difference between drug and control means ± S.E.	Mean	Difference between drug and control means ± S.E.
2. Lee-White (silicone) (minutes)	22·25	23·05	+0·80 ± 1·36	22·30	+0·05 ± 1·36
6. "Prothrombin time" (venom thromboplastin) (seconds) ..	14·87	15·11	+0·24 ± 0·20	15·00	+0·13 ± 0·20
7. Thrombin clotting time (seconds)	16·19	15·68	−0·51 ± 0·50	16·40	+0·21 ± 0·50
8. Waugh-Ruddick (heparin) (minutes/u. heparin)‖	60·00	35·10	−24·90 ± 13·05	64·80	+4·80 ± 13·05
9. Platelet count (thousands/cu. mm.) ..	325	331	+6 ±13	328	+3 ±13

The pooled means for the adrenaline and saline tests have been used as control values. No differences are significant.

‖ Results are expressed as the slope of the regression of clotting time on heparin concentration.

S.E. = Standard Error of the difference between the means.

Note that for an analysis with no missing readings there are only 12 degrees of freedom for error: where $n = 12$, $t = 2{\cdot}179$ for $P = 0{\cdot}05$.

5. *Differences between subjects and between days.* A number of significant differences were detected between mean results obtained from different subjects and on different days, but the design of these experiments was not suitable for studying the magnitudes of these differences and they are therefore not presented.

TABLE VIIIB

EXPERIMENT 3. THE EFFECTS OF PENICILLIN AND OF STREPTOMYCIN

Mean clotting times (seconds) for the brain "prothrombin time", to show significant differences.

Controls			Penicillin tests (immediate)		Streptomycin tests (immediate)	
Saline	Adrenaline					
Mean	Mean	Difference between drug and saline means ± S.E.	Mean	Difference between drug and saline means ± S.E.	Mean	Difference between drug and saline means ± S.E.
15·31	14·64	−0·67* ± 0·24	14·56	−0·75** ± 0·24	14·94	−0·37 ± 0·24

* Difference significant at the 5 per cent. level.

** Difference significant at the 1 per cent. level.

S.E. = Standard Error of the difference between the means.

Note that for an analysis with no missing readings there are only 12 degrees of freedom for error: where $n = 12$, $t = 2{\cdot}179$ for $P = 0{\cdot}05$.

6. *Residual error variances: the experimental error of the tests.* In tests where replicate readings have been obtained, the error component of the residual variance can be directly determined, and the experimental error of the different tests may be usefully compared by means of the coefficient of variation. These data are shown in Table IX.

DISCUSSION

1. *Effects due to Penicillin and to Streptomycin*

(i) *Clotting time tests.* In certain tests the clotting times appear to have been influenced by penicillin and by streptomycin, but the irregular

TABLE IX

EXPERIMENTAL ERRORS: Approximate values for the standard deviations for experimental error have been obtained from the ranges observed within replicates: for purposes of comparison these values have been expressed as percentages of the corresponding means

Test	Coefficients of variation per cent. (from scatter within replicates)				
	Experiment 1 (penicillin)	Experiment 2 (streptomycin)	Experiment 3 (both drugs)	Unweighted mean (present 3 experiments)	Estimates of Briggs and MacMillan (1948)
1. Lee-White (glass)	14·7	11·3		13·0	7·0
2. Lee-White (silicone)	15·9	20·2	13·0	16·4	
3. Dale-Laidlaw	9·3	10·8		10·1	25·1
4. "Prothrombin consumption"	7·0	7·8		7·4	
5. "Prothrombin time" (brain thromboplastin)	2·0	4·4	5·2*	3·9	
6. "Prothrombin time" (venom thromboplastin)	3·0	2·6*	1·7	2·4	
7. Thrombin clotting time	3·7	4·9	6·5*	5·0	

* In these tests a different observer recorded the results in the indicated instances: in the other tests observations were mostly obtained by the same observer(s).

pattern of the significant effects is bizarre. Moreover, individual significant effects were of the order of only a 10 per cent. increase or decrease over control values. It therefore seems wise to ascribe no fundamental importance to any of these effects: where a number of tests are made an occasional "significant" effect of low magnitude must be expected to arise by chance. Hence this work does not support the earlier claims of a coagulant effect of penicillin and of streptomycin of which Macht[20,21,22,23] has been the chief exponent.

Macht's (*passim*) studies are difficult to discuss. There is no doubt of the marked effects on clotting time of these and many other drugs apparent in his published data. Nevertheless his results may fairly be questioned in the many instances in which he has not used concurrent controls but has compared the clotting times obtained after the administration of a drug with those previously obtained from the same subject. In such circumstances it is always possible that the shortening in clotting time is due to some other cause, such as the progress of anæsthesia, the effect of restraint in the unanæsthetised animal, the method of administration of the drug or even to the effect of obtaining serial blood samples. For instance, a shortening of the clotting time following emotional reaction in the cat was demonstrated by Cannon and Mendenhall[24] and a similar effect in animals and in man has recently been studied by Macht[25] himself. Menghini and Giunti[26] have observed in man a progressive shortening of clotting time in serial venepunctures without the administration of any drug.

(ii) *Plasma fibrinogen determination.* This measurement is probably liable to less subjective error than the observations of clotting time. It is therefore reasonable to look for a biological explanation of the 20 per cent. rise in the delayed streptomycin value over control, significant at the 5 per cent. level, in Experiment 2 (Table VII). This might be due to a mild toxic action of the drug: a rise in the plasma fibrinogen concentration is a sensitive indication of toxic or inflammatory processes (Foster and Whipple[27]; Gram[28]; Schultz *et al.*[29]) and the body might respond in this way to streptomycin before clinical evidence of toxicity was apparent. A subclinical toxic action of streptomycin has also been detected electrocardiographically by di Maria.[30]

2. *Residual Error Variances*

Table IX shows great differences between the error coefficients of the various tests. The heparin resistance test showed the greatest variability and the plasma tests the least.

Biggs and MacMillan[31] obtained estimates of experimental error for whole blood clotting times (Lee-White glass and Dale-Laidlaw) in their detailed study of laboratory errors. Their estimates are based on differences between replicates, with no interaction; their values are included in Table IX for comparison.

Summary

1. The effects of penicillin and of streptomycin on blood coagulation have been studied on 20 normal subjects after injection of the drugs.

Each subject acted in turn as "test" and "control" and 3 experiments were made: 2, each using 6 subjects, on the immediate and delayed effects of the 2 drugs separately, and 1, using 8 subjects, on the immediate effects of both drugs. Symmetrical experimental designs eliminated differences between subjects and between days of test. 9 tests of clotting function were used, in addition to the counting of platelets and the determination of plasma fibrinogen.

2. A few significant differences were detected between test and control subjects but no systematic effect was apparent through all 3 experiments.

3. Estimates are given for the experimental error of the various tests. The error was relatively low for one-stage "prothrombin times" and thrombin clotting times, and relatively high for whole-blood-clotting times.

4. No support has been obtained for previous work reporting a coagulant action of penicillin and streptomycin.

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REFERENCES

1. Frada, *Giorn. Med., Palermo*, 1946, **3**, 95.
2. Courty and Biscaye, *Presse méd.*, 1947, **55**, 734.
3. Ochsner, Kay, DeCamp, Hutton and Balla, *Ann. Surg.*, 1950, **131**, 652.
4. Hines and Kessler, *J. Amer. med. Ass.*, 1945, **128**, 794.
5. Lee and White, *Amer. J. med. Sci.*, 1913, **145**, 495.
6. Dale and Laidlaw, *J. Path. Bact.*, 1911–12, **16**, 351.
7. Biggs and Macfarlane, *J. clin. Path.*, 1949, 2, 33.
8. Waugh and Ruddick, *Canad. med. Ass. J.*, 1944, **50**, 547.
9. Waugh and Ruddick, *ibid.*, 1944, **51**, 11.
10. de Takats, *Surg. Gynec. Obstet.*, 1943, **77**, 31.
11. Ingram, *Biochem. J.*, 1952, **51**, 583.
12. Baar, *J. clin. Path.*, 1948, **1**, 175.
13. Waldron and Friedman, *Fed. Proc.*, 1948, **7**, 130.
14. Stewart and Hendry, *Biochem. J.*, 1935, **29**, 1677.
15. Sackett, *J. biol. Chem.*, 1925, **64**, 203.
16. Schoenheimer and Sperry, *ibid.*, 1934, **106**, 745.
17. Stewart and Hendry, *Biochem. J.*, 1935, **29**, 1683.
18. Silverman, *Blood*, 1948, **3**, 147.
19. Hagedorn and Jensen, *Biochem. Z.*, 1923, **135**, 46; **137**, 92.
20. Macht, *Arch. int. Pharmacodyn.*, 1947, **74**, 399.
21. Macht, *Science*, 1947, **105**, 313.
22. Macht, *Southern med. J., Nashville*, 1948, **41**, 720.
23. Macht and Ostro, *Science*, 1946, **103**, 402.
24. Cannon and Mendenhall, *Amer. J. Physiol.*, 1914, **34**, 251.
25. Macht, *Fed. Proc.*, 1951, **10**, 88.
26. Menghini and Giunti, *Rass. int. Clin. Terap.*, 1948, **28**, 421.
27. Foster and Whipple, *Amer. J. Physiol.*, 1921–22, **58**, 407.
28. Gram, *Acta med. scand.*, 1922, **56**, 107.
29. Schultz, Nicholes and Schaefer, *Amer. J. Path.*, 1925, **1**, 101.
30. di Maria, *Minerva med., Torino*, 1951, **42**, i, 683.

31. Biggs and MacMillan, *J. clin. Path.*, 1948, **1**, 269.
32. Moldavsky, Hasselbrock and Cateno, *Science*, 1945, **102**, 38.
33. Ungar, *Lancet*, 1945, **249**, 614.
34. Lewis, *Proc. Soc. exp. Biol., N.Y.*, 1946, **63**, 538.
35. Weiner, Zeltmacher and Shapiro, *Exp. Med. Surg.*, 1948, **6**, 181.
36. Dolkart, Halpern, Larkin, Dey and de Takats, *J. Pharmacol.*, 1949, **96**, 291.
37. Reggianini, *Boll. Soc. ital. Biol. sper.*, 1949, **25**, 222.
38. Donatelli and Pasquinucci, *ibid.*, 1948, **24**, 963.
39. Meneghini, Cataldi and Gozzano, *Arch. E. Maragliano*, 1950, **5**, 657.
40. Giannico and Provini, *Clin. nuova*, 1949, **8**, 232.
41. Farrington, Hull-Smith, Bunn and McDermott, *J. Amer. med. Ass.*, 1947, **134**, 679.
42. Nassi and Ulivelli, *Boll. Soc. ital. Biol. sper.*, 1948, **24**, 956.
43. de Michele and Portella, *Rass. int. Clin. Terap.*, 1949, **29**, 103, 222.
44. Elson, *Amer. J. med. Sci.*, 1949, **217**, 42.

THE TOXICITY OF THE DINITRO-CRESOLS

PART I. 4:6 DINITRO-*ortho*-CRESOL AND ITS SIMPLER DERIVATIVES

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INTRODUCTION

4:6-Dinitro-*o*-cresol* is widely used as an ovicide in winter washes for fruit trees and in the spring and early summer for the control of weeds in corn (David[1]). A recent report also indicates that it may prove to be effective as an anti-locust measure.[2] Attention has been drawn to the dangers likely to be encountered in its use (Bidstrup and Payne[3]) and to the fact that it acts as a cumulative poison in man (Harvey, Bidstrup and Bonnell[4]; Bidstrup, Bonnell and Harvey[5]). Suggestions have been made that its toxicity may be complicated by the presence of isomeric dinitro-cresols that may be formed during the course of its manufacture. This theory has also been advanced by Molnar[6] who has described extensive toxicological tests on two isomeric dinitro-*o*-cresols. Although one of these has properties that suggests that it is probably identical with 4:6-dinitro-*o*-cresol Molnar has neither specified the structure nor detailed the manufacturing processes of either compound. A discussion of this aspect will be included in a later communication.

The present paper deals with a general survey of the physical properties and toxicity of some commercial samples of 4:6-dinitro-*o*-cresol and their simpler derivatives which have been manufactured in the United Kingdom.

MANUFACTURE

A process at present employed is essentially that described by Nolting and Salis.[8] High grade *o*-cresol is sulphonated with sufficient sulphuric acid to give the 4:6-disulphonate derivative. The mixture containing this substance is then treated with nitric acid at a temperature above the melting point of the nitro-compound. This ensures complete nitration. The crude nitro-compound is separated from the acid mixture while still in the molten state. This reduces the amount of occluded impurities. The crude product is washed with cold water to remove the last traces of mineral acids. It is then packed in drums while still in the moist state. This is then ready for despatch. Most solutions are made up as ammonium or sodium salts since these are more soluble than dinitro-*o*-cresol itself.

PHYSICAL PROPERTIES OF SOME COMMERCIAL SAMPLES

The moisture content, solubility at 18° C., melting points and mixed melting points with pure samples were determined in the usual way. 7

* An alternative name is 3:5-dinitro-*o*-cresol. This is used by Parker, Barnes and Denz.[7]

commercial samples of dinitro-*o*-cresol and 1 each of the sodium, ammonium and diethylamine salts were thus examined. In addition a sample of 4:6-dinitro-*sec.*-butyl phenol was included in the survey since this substance is also used in spray operations, although not to the same extent as dinitro-*o*-cresol. Table I lists the results of the examinations.

In view of the possibility of the occurrence of additional isomers or toxic impurities an attempt was made to remove them on an alumina

TABLE I

PHYSICAL PROPERTIES OF SOME COMMERCIAL SAMPLES OF 4:6-DINITRO-*o*-CRESOL AND ITS DERIVATIVES

Sample	Mol. wt.	Descriptions index letter	Colour	Moisture per cent.	Solubility H_2O(18°C.)	Melting point °C. (*a*)	Mixed melting point °C.	Recovery of dinitro-*o*-cresol in 1 per cent. solution Theory 1 per cent. (*b*)
4:6-Dinitro-*o*-cresol	198	Pure recryst.	Bright yellow	nil	0·024	86·0	—	0·96
		A	Pale brown	15·0	0·018	86 to 88	86 to 86·5	0·99
		B	Yellow brown	10·7	0·022	84 to 86	82 to 86	0·88
		C	Yellow	0·07	0·017	86 to 88	85 to 89	1·00
		D	Pale brown	6·0	0·021	83 to 86	84 to 86	1·00
		E	Brown	4·0	0·021	84 to 87	83 to 89	0·99
		H	Pale brown	7·9	0·017	84 to 88	85 to 89	0·99
		K	Yellow	9·9	0·018	84 to 87	82 to 89	1·00
Ammonium .. Salt	214 (+8·5)	Pure recryst.	Bright yellow	nil	0·91	Decomp.	—	0·88 (−8·3) (*c*)
		G	Bright yellow brown	1·3	0·98	Decomp.	Decomp.	0·88
Diethylamine .. Salt	272 (+37)	Pure recryst.	Yellow	nil	7·4	110 to 111	—	0·73 (−25) (*c*)
		F	Yellow	4·7	7·5	108 to 110	93 to 106	0·69
Sodium salt ..	220 (+11)	J	Golden yellow	21·4	7·9	Decomp.	—	0·79 (−17) (*c*)
4:6-Dinitro-*sec.*-butylphenol (*d*)	240 (+21)	95 per cent. pure	Yellow	nil	0·004	45	45	0·75 (−22) (*c*)
		Pure recryst.	Yellow	nil	0·006	45		0·80

NOTES.

(*a*) Melting points uncorrected. Material dried *in vacuo* over sulphuric acid before use. Heilbron and Bunbury[9] give 86·5° as m.pt. of pure 4:6-dinitro-*o*-cresol.

(*b*) 1·00 g. dissolved in slight excess of aqueous sodium bicarbonate and the solution made up to 100 ml.

(*c*) The reduction in the amount of dinitro-*o*-cresol estimated by Parker's method[10] is approximately equivalent to the increase in the molecular weights of the salts and consequent diminution of "active" compound per unit volume of solution.

(*d*) 4-6-Dinitro-*sec.*-butylphenol has similar chemical and physiological properties to 4:6-dinitro-*o*-cresol. It can also be estimated by the method of Parker (*loc cit.*)

column. With the exception of a small quantity of dark brown amorphous benzene insoluble material no substances other than 4:6-dinitro-*o*-cresol could be isolated from the samples examined. Relative purity of the substances examined was also expected from the small depressions obtained from the mixed melting points.

Toxicity Tests

All solutions of the samples were made up to 1 per cent. w/v by dissolving exactly 1·0 g. of the material (dried at 60° C. and then over sulphuric acid *in vacuo*) in 1·0 per cent. saline solution containing 1·0 per cent. of sodium bicarbonate and making up to 100 ml. The solutions were administered by subcutaneous injection. Pure recrystallised 4:6-dinitro-*o*-cresol was made up as a 1 per cent. solution in a similar manner and used as a reference throughout. Pilot experiments were carried out to determine the approximate LD50 on small groups of about 4 rats per group. Thereafter 10 animals were used per dose level. 2 or 3 dose levels were selected, the mortalities over 24 hours converted into probit values which were plotted against log doses, and the LD50 calculated in the usual way.

In view of the importance of the alimentary canal as route of absorption of dinitro-*o*-cresol and the possible effect of heat in stimulating its metabolic effects (Bidstrup and Payne, *loc. cit.*, Barnes *et al. loc. cit.*, King and Harvey[11]) a further simple comparative test was carried out. This consisted of administering by stomach tube a single sub-lethal dose to rats and noting their response and mortality to 4 hours exposure at 20° to 22° C., 5 hours at 37° to 40° C. and 15 hours at 20° to 22° C. The dose level was 50 mg./kg., and the samples were dissolved in aqueous bicarbonate solution to give 1 per cent. strength. 10 rats were used per sample. The results of both toxicity tests are given in Table II.

Discussion

The evidence presented indicates that the commercial samples that have been examined are slightly less toxic than the pure compound and that the ammonium, sodium and diethylamine salts are also less toxic than dinitro-*o*-cresol. Also that 4:6-dinitro-*sec*.-butylphenol is slightly more toxic than dinitro-*o*-cresol. Indirectly this also suggests that if the manufacturing process is carried out in general agreement with the method of Nolting and Salis, using high grade *o*-cresol uncontaminated by the *meta* and *para* isomers there will be little likelihood of any other dinitro-cresols or by-products being formed in any quantity.

The two values obtained for the LD50 on the two series of rats are in fairly close agreement with the values quoted by Ambrose,[12] 25 mg./kg., and by Parker *et al.* (*loc. cit.*), 24·6 mg./kg.

Although the diethylamine salt is nearly half as toxic as pure dinitro-*o*-cresol this can probably be correlated with the increased molecular weight and the consequent reduction of the amount of "active" compound per unit volume of its solution in water or other solvent. It is possible that the decreased toxicity and the increased solubility of this salt may have

some practical bearing on its use in the field. In the first place comparison of the "effective" quantities of the diethylamine salt and other derivatives required for weed killing suggests that less of the former compound may be required.

The second point is that the increased solubility of the diethylamine salt aids its dispersion since it does not sediment readily in the storage tanks and will not block the nozzles in the spray beam. This means that the

TABLE II

TOXICITY OF SOME COMMERCIAL SAMPLES OF 4:6-DINITRO-*o*-CRESOL AND THEIR DERIVATIVES

Substance	Subcutaneous injection LD50		Stomach tube, mortality out of 10			
	1st series (albino rats)	2nd series (hooded rats)	4 hours 20° to 22° C.	5 hours 37° to 40° C.	15 hours 20° to 22° C.	Total per cent.
4:6-Dinitro-*o*-cresol pure ..	25·6	28·5	0	7	0	70
,, Comm. A	26·2	—	3	7	0	100
,, B	26·8	—	3	7	0	100
,, C	27·5	—	2	8	0	100
,, D	—	—	0	9	0	90
,, E	—	—	0	10	0	100
,, H	26·8	—	0	9	0	90
,, K	—	—	1	7	0	80
Ammonium salt, pure	—	—	0	6	1	70
Comm. G ..	—	27·5, 30·0	0	10	0	100
Diethylamine salt, pure	—	36·5, 39·1	0	1	2	30
Comm. F	—	—	0	8	0	80
Sodium salt (*a*) ..	—	—	0	9	0	90
Comm. J, 4:6-dinitro-*sec*-butyl phenol, 95 per cent.	—	21·4	0	6	4	100

NOTE.—(*a*) This was not assayed as all other samples of 4:6-dinitro-*o*-cresol were administered as solutions of their sodium salts.

spray operator will not need to clear the nozzles and therefore will not incur the risk of a sudden shower of spray (cf. Bidstrup and Payne[3]). In spite of these practical points, which are of obvious importance, it must be remembered that increased solubility may increase absorption and every effort must be made to continue protective devices.

From this study it can be concluded that all the dinitro-*o*-cresol compounds which have been investigated are dangerous. Although the diethylamine salt is less toxic than the other compounds studied it appears to have similar physiological effects at normal and elevated environmental temperatures. The lower toxicity of the diethylamine salt suggests a profitable line of research in finding other derivatives of dinitro-*o*-cresol which, even if they have the same qualitative effect may be quantitatively less active as human poisons.

SUMMARY

1. Commercial samples of dinitro-*o*-cresol and their common salts have been examined by physical and toxicological tests.

2. Commercial samples of dinitro-*o*-cresol and its salts are less toxic than the pure substance.

3. The diethylamine salt is about 50 per cent. less toxic than pure 4:6-dinitro-*o*-cresol.

4. 4:6-Dinitro-*sec.*-butylphenol is slightly more toxic than 4:6-dinitro-*o*-cresol.

The author wishes to thank the several commercial firms who have kindly supplied him with samples of 4:6-dinitro-*o*-cresol and its salts, and who have assisted him with much useful information; also Professor V. H. Blackman for a sample of 4:6-dinitro-*sec.*-butylphenol; Dr. Donald Hunter, Head of the Department, for encouragement; Dr. P. Lesley Bidstrup and Dr. J. A. Bonnell for valuable discussions; Dr. F. J. Dyer for much advice and help, and finally Miss Jean Peal, Mr. K. E. Carling and Miss A. Mackrill for technical assistance.

References

1. David, *Trans. Assoc. Ind. Med. Officers*, 1951, **1**, 14.
2. *The Times*, *Special article*, *Red Locusts in N. Rhodesia*, 12th Feb., 1952.
3. Bidstrup and Payne, *Brit. med. J.*, 1951, **2**, 16.
4. Harvey, Bidstrup and Bonnell, *Brit. med. J.*, 1951, **2**, 13.
5. Bidstrup, Bonnell and Harvey, *Lancet*, 1952, **262**, 794.
6. Molnar, *Ann. Physiol.*, 1937, **13**, 1164.
7. Parker, Barnes and Denz, *Brit. J. Ind. Med.*, 1952, **8**, 226.
8. Nolting and Salis, *Ber. dtsch. chem. Ges.*, 1881, **14**, 987.
9. Heilbron and Bunbury, *Dictionary of Organic Compounds*, Vol. 1, 1946, 983.
10. Parker, *Analyst*, 1949, **74**, 646.
11. King and Harvey, 1952, *unpublished results*.
12. Ambrose, *J. Pharmacol.*, 1942, **76**, 245.

THE PHOTOMETRIC DETERMINATION OF 2-AMINO-5-NITROTHIAZOLE AND CERTAIN ACYL DERIVATIVES

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The use of 2-amino-5-nitrothiazole in veterinary medicine makes it desirable to have available a method for its determination in aqueous solution and in blood. Although diazotisation of aminothiazoles in hydrochloric acid yields chlorine substituted products,[1] in sulphuric acid the normal diazo-salt is formed. Optimum conditions were, therefore, established for diazotisation of aminonitrothiazole in sulphuric acid followed by coupling with *N*-naphthylethylenediamine and a method for the determination of aminonitrothiazole in aqueous solution was evolved. This method was applicable to the phthalyl and succinyl derivatives after hydrolysis and to blood after a suitable deproteinisation procedure.

Proposed Method

Reagents

(1) Sulphuric acid 20N (55 per cent. v/v).
(2) Sodium nitrite solution 2 per cent.
(3) Sulphamic acid solution 2·5 per cent.
(4) *N*-naphthylethylenediamine hydrochloride solution 0·4 per cent.
(5) Trichloracetic acid 10 per cent.

Procedures

(*a*) *For simple solutions of aminonitrothiazole.* Into a 25-ml. flask, pipette 1 ml. of a solution containing up to 0·5 mg. of aminonitrothiazole in dilute sulphuric acid, add 5 ml. of 20N sulphuric acid and cool in ice-water for 2 minutes. Add 1 ml. of 2 per cent. sodium nitrite, mix and leave for 4 minutes; add 1 ml. of 2·5 per cent. sulphamic acid, mix and leave for 4 minutes. Add 1 ml. of 0·4 per cent. *N*-naphthylethylenediamine hydrochloride solution, mix and leave for 2 minutes. Remove the flask from the ice bath, dilute to 25 ml. with ethanol (95 per cent.) and within 5 minutes of dilution measure the extinction of 1 cm. at 580 mμ. or using a suitable filter. Read off the amount of aminonitrothiazole from a standard curve prepared using pure aminonitrothiazole. Calibration data are given in Table I.

(*b*) *For succinylaminonitrothiazole.* Pipette 1 ml. of solution containing up to 0·8 mg. of succinylaminonitrothiazole into a 25-ml. flask, add 5 ml. of 20N sulphuric acid and heat in a boiling water bath for 40 minutes. Cool in ice-water for 5 minutes and proceed as in (*a*) commencing with the words "Add 1 ml. of 2 per cent. sodium nitrite solution." Read off the amount of succinylaminonitrothiazole from a standard curve prepared by this method, using pure succinylaminonitrothiazole.

(*c*) *For phthalylaminonitrothiazole.* Proceed as for succinylamino-nitrothiazole, but heat for 90 minutes at 100° C. instead of for 40 minutes.

(*d*) *For aminonitrothiazole and acyl derivatives in blood.* To 1 ml. of blood in a small centrifuge tube add 2 ml. of water and mix, add 1 ml. of 10 per cent. trichloracetic acid, mix well and centrifuge for 2 minutes. If aminonitrothiazole is present, pipette 1 ml. of the supernatant liquid into a 25 ml. flask, add 5 ml. of 20N sulphuric acid and continue as in (*a*), commencing with the words "cool in ice/water for 2 minutes." If succinyl- or phthalyl-aminonitrothiazole is present, treat 1 ml. of solution as in (*b*) and (*c*) respectively.

Notes on the Method

Procedure (*a*). Variation in acidity affects the tint but not the intensity of the final colour. The purpose of the ethanol is to prevent precipitation of the pigment and minimise fading which, in the final method, is at the rate of about 1 per cent. in 3 minutes. The reproducibility of the method is illustrated by the following values obtained in determinations carried out in pairs at different times; 0·810, 0·810; 0·800, 0·810; 0·813, 0·800; 0·815, 0·815; 0·810, 0·805.

TABLE I

CALIBRATION DATA FOR THE DETERMINATION OF AMINONITROTHIAZOLE IN AQUEOUS SOLUTION

Aminonitrothiazole mg.	Extinction of 1 cm. at 580 mμ	Slope
0·05	0·165	3·3
0·10	0·335	3·4
0·15	0·505	3·4
0·20	0·65	3·3
0·25	0·77	3·1
0·30	0·88	3·0
0·35	1·01	2·9
0·40	1·145	2·9
0·45	1·26	2·8

Procedures (*b*) *and* (*c*). Under the conditions used for the hydrolysis of phthalyl- and succinyl-aminonitrothiazole, hydrolysis to aminonitrothiazole is complete and no decomposition of the latter occurs.

Procedure (*d*). When determinations were made on blood to which known amounts of aminonitrothiazole had been added, recovery was 70 per cent. owing to the adsorption which commonly occurs in deproteinisation. Allowance may be made for this either by applying a correction or by using a calibration curve based on data obtained by applying the method to aminonitrothiazole in the presence of blood.

SUMMARY

1. A photometric method, based on diazotisation and coupling, for the determination of aminonitrothiazole and its phthalyl- and succinyl-derivatives is proposed.

2. The method is applicable to blood after the deproteinisation procedure described.

Thanks are due to the directors of May and Baker, Ltd., for permission to publish this paper.

REFERENCE

1. Morgan and Morrow, *J. chem. Soc.*, 1915, **107**, 1291.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

CHEMISTRY

ALKALOIDS

Ergot Alkaloids, Paper Chromatography of. V. E. Tyler and A. E. Schwarting. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 354). Descending paper partition chromatography (22·5 in Whatman No. 1 paper) was used. Filter paper strips were impregnated with propylene glycol, and formamide, and after application of the alkaloids, the wash liquids of benzene, toluene, and xylene, each equilibrated with the stationary-phase liquid, were used to form the chromatograms; separations of the water-insoluble alkaloids were possible, but not adaptable to all of the alkaloids. Hydrophobic filter paper was prepared by dipping strips or sheets of paper in a 5 per cent. v/v solution of Dow-Corning Silicone No. 1107 in heptane, allowing to dry in air, and heating in an oven at 150° C. for 3 minutes. After application of the alkaloids the paper was equilibrated for 12 hours with the vapour of the saturated butanol phase of the wash liquids, the chromatograms were formed with the saturated aqueous phase of the several wash liquids. The aqueous phases of the butanol-acetic acid-water and butanol-*p*H 3·0 buffer systems gave the broader separations; R_F values of the alkaloids obtained with these two systems are reported. The methods fail in the separation of ergotoxine and its isomer; ergotoxine and ergotinine are separable when chromatographed with a butanol *p*H 4·5 buffer system, the R_F of ergotoxine being 0·60 and R_F of ergotinine 0·69. R. E. S.

Veralbidine, A New Alkaloid from *Veratrum album*. A. Stoll and E. Seebeck. (*Science*, 1952, **115**, 678). From the mother liquors obtained in the isolation of protoveratrine, jervine, and rubijervine, a new alkaloid "veralbidine" was separated. The pure substance crystallises from dilute acetone in pentagonal plates, from dilute methanol in prisms, and from ether in fine needles. It melts between 181° and 183° C. and has $[\alpha]_D^{20°\,C.} = -11{\cdot}7°$ in pyridine and $+5{\cdot}4°$ in chloroform. In 84 per cent. sulphuric acid, veralbidine gives a colourless solution. It is sparingly soluble in ether, ethanol, and acetone, insoluble in water but readily soluble in chloroform. It is irritating to the nasal mucosa, causing sneezing. The empirical formula is $C_{37}H_{61}O_{12}N$. Veralbidine yields a crystalline thiocyanate $C_{37}H_{61}O_{12}N,HNCS$ which melts at 235° to 236° C. with decomposition and frothing and a crystalline hydrochloride $C_{37}H_{61}O_{12}N,HCl$ readily soluble in ethanol and water and melting at 250° to 251° C. R. E. S.

ANALYTICAL

Alcohols, Colorimetric Determination of. V. W. Reid and R. K. Truelove. (*Analyst*, 1952, 77, 325). The use of ceric ammonium nitrate as a colorimetric reagent for the quantitative determination of various alcohols in dilute aqueous solutions is described. A standardised reagent is used and after mixing with the sample the colour is measured after exactly 5 minutes as the colours produced with lower alcohols are unstable. Calibration data for methanol, ethanol, *iso*propanol, *n*-butanol, *sec.*-butanol, *tert.*-butanol, monoethylene

glycol and triethylene glycol are given. Tertiary alcohols, in contrast to primary and secondary alcohols, produced a colour of excellent stability almost directly proportional to the concentration. Certain polyhydric alcohols gave stable colours, but others either no coloration or unstable colours. Sucrose gave an excellent calibration curve, whereas dextrose did not. Glycerol produced deep unstable colours in dilute solutions. Certain oxidising agents (such as organic peroxides), reducing agents, sulphate ions and other compounds containing hydroxyl groups interfered; methods for the separation of interfering substances are given. The application of the method to the analysis of effluents and to the determination of trace amounts of alcohol is discussed.

R. E. S.

2:8-Diaminoacridines, Colorimetric Determination of. J. G. Devi and M. L. Khorana. (*Indian J. Pharm.*, 1952, **14**, 43). A method is described for the assay of proflavine sulphate and hemi-sulphate. The substance in solution is diazotised and the diazotised product is coupled with resorcinol in an alkaline solution; the intensity of the colour developed is then compared with a similarly treated standard solution. Although alkaline solutions of resorcinol darkened on standing and had to be freshly prepared, this substance produced a stable intense red colour on diazotization which possessed advantages over the colours yielded by α- and β-naphthols, phoroglucinol, thymol and guaiacol. The colour produced with resorcinol showed a broad spectrum maximum at 544 to 548 mμ and could be measured photoelectrically; the colour intensity obeyed Beer's Law and experiments showed that it was stable up to 3 hours when kept at a low temperature; at room temperature the value for the blank changed quite rapidly. It is claimed that the results indicated the method to be as accurate as the official B.P. method. R. E. S.

Digitalis Glycosides, Chemical Determination of. M. Kåern. (*Dansk Tidsskr. farm.*, 1952, **26**, 89.) A mixture of digitoxin and digitoxigenin can be assayed by the following process. 5 ml. of a chloroform solution, containing 0·25 to 0·5 mg. of digitoxin, is evaporated to dryness and the residue is dried for 30 minutes at 100° C. The residue is dissolved in 5 ml. of ethanol and the digitonin is determined colorimetrically (see below). Another 5 ml. of the original solution is passed through a column of alumina (2 g. in 0·5 cm. diameter tube) and eluted with 3 quantities, each of 5 ml., of chloroform. This solution is assayed for digitoxigenin. The column is then again eluted with 3 quantities, each of 5 ml., of a mixture of 3 parts of chloroform and 1 part of ethanol, to give a solution containing the digitoxin. For the colorimetric assay 5 ml. of a methanol solution is mixed with 5 ml. of Knudson-Dresbach reagent (0·95 g. of picric acid and 5 ml. of 10 per cent. sodium hydroxide solution in 100 ml.). The colour is measured within 15 to 30 minutes in a 1 cm. cell at 492 mμ. The factor for conversion of E to digitoxin (in 10 ml. of reaction mixture) is $1{\cdot}07 \times 10^{-2}$; and for digitoxigenin $2{\cdot}21 \times 10^{-2}$. The method may be applied to digitoxin tablets, but not to tincture of digitalis. G. M.

Galenical Preparations, Identification of, by Paper Chromatography. A. B. Svendsen. (*Dansk. Tidsskr. farm.*, 1952, **26**, 125.) Strip paper chromatography may be applied to the identification of galenicals. Examples are given of the application to morphine preparations, the solvent being prepared by shaking together 10 volumes of butanol, 2 of glacial acetic acid, and 10 of water, and, after separation, using the butanol layer. For development either nitrite reagent or potassium bismuth iodide solution is used. Morphine

solutions may be tested directly, using the equivalent of about 100μg. of the salt. For injection of scopolamine, morphine and ephedrine, 0·01 ml. of the solution is run in the usual way, and developed successively with potassium bismuth iodide (morphine + scopolamine), nitrite (= morphine) and ninhydrin (ephedrine). In the case of tetrapon injection the presence of glycerin interferes with the chromatography, since the glycerin itself accumulates at an R_F of about 0·45 and interferes with the morphine and codeine, although not with narcotine and papaverine. Thus nitrite reagent gives a long streak which has a weaker colour in the middle, where the glycerin is concentrated. In tincture of opium the morphine can easily be detected. For morphine suppositories it is necessary to prepare an extract by shaking with warm water; and for opium suppositories by shaking with water containing 1 per cent. of hydrochloric acid.

G. M.

Histamine in Pharmaceutical Products, Determination by means of 2:4-Dinitrofluorobenzene. F. C. McIntire. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 277.) A test solution is prepared to contain 20 to 100 μg. of histamine base per ml. in 0·004M sodium diethylthiocarbamate buffered with a concentration of 0·1M sodium carbonate and 0·1M sodium bicarbonate. The solvent is distilled water of low copper content. 1 ml. of a 0·6 per cent. v/v solution of 2:4-dinitrofluorobenzene is mixed with 1 ml. of the test solution, allowed to stand for 20 minutes and diluted to 20 ml. with 0·1N hydrochloric acid. The solution is extracted with benzene and the optical density of the aqueous phase measured at 358 mμ, the histamine concentration being calculated from the data for pure histamine. A reaction blank test and 2 different concentrations of a histamine standard should be included in each set of determinations. The method has a high degree of precision and reproducibility. Solutions containing a high proportion of phenol as a preservative should be submitted to a preliminary extraction with benzene or ether to remove most of the phenol which may interfere in the reaction. G. B.

***iso*Nicotinyl Hydrazide and γ-Picoline, Photometric Determination of.** C. W. Ballard and P. G. W. Scott. (*Chem. Ind.*, 1952, 715). The method is based on the fact that a purple colour with 1-chloro-2:4-dinitrobenzene is given by γ-picoline and *iso*nicotinyl hydrazide but not by 2:6-lutidine, α-picoline, β-picoline, pyridine, *iso*nicotinic acid or ethyl *iso*nicotinate. To 5 ml. of a solution in dehydrated ethanol containing up to about 0·1 mg. of γ-picoline or *iso*nicotinyl hydrazide is added, 5 ml. of a 5 per cent. solution of 1-chloro-2:4-dinitrobenzene in dehydrated ethanol and 0·1 g. of borax; after heating in a boiling water bath for 10 minutes, cooling and adding 25 ml. of methanol, the mixture is filtered and the optical density is measured at 560 mμ (γ-picoline) or 530 mμ (*iso*nicotinyl hydrazide). Calibration curves are almost linear and reproducibility is about ± 2 per cent. for γ-picoline and ± 3 per cent. for *iso*nicotinyl hydrazide. Up to 0·2 ml. of water may be present in a determination without appreciable effect. The colour fades in intense artificial light or bright daylight but not in weak artificial light or in ultra-violet light. The method can be used for the determination of γ-picoline in mixtures of picolines containing pyridine and for the determination of *iso*nicotinyl hydrazide in pharmaceutical preparations and, possibly, biological fluids. R. E. S.

Oxalates in Fresh Plant Material, Determination of. C. J. L. Baker. (*Analyst*, 1952, **77**, 340). A method is described for determining total oxalates in plants, by extraction with hydrochloric acid, precipitation as calcium oxalate

from the deproteinised extract and subsequent titration with potassium permanganate; removal of proteins is accomplished with a sodium tungstate-phosphoric acid reagent. Water-soluble oxalates are determined similarly on an aqueous extract. In each case the oxalate in the final stages is precipitated with calcium chloride, the precipitate being cooled overnight in the refrigerator, separated by centrifuging and then heated with dilute sulphuric acid before titration with permanganate. The efficiency of the extraction method was examined by studying the variation in oxalate content of the extract with length of time in contact with the solid material; results showed that extraction was complete after 16 hours. The method is designed for fresh green plants only, as oxalate is lost on drying the material. R. E. S.

Pilocarpine, Colorimetric Assay of. J. W. Webb, R. S. Kelley and A. J. McBay. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 278.) The following method is applicable to the determination of pilocarpine and its salts even in low concentrations. It is based on the method of Shupe, but avoids the liberation of the unstable free base during the process. To solutions containing 2 to 6 mg. in 5 ml. of water, a mixture of 1 ml. of acetic acid (20 per cent.), 10 ml. of chloroform, 1 ml. of a 5 per cent. solution of potassium chromate and 2 ml. of a 3 per cent. solution of hydrogen peroxide is added, and the liquid shaken in a separator. The chloroform solution is separated and the aqueous solution extracted successively with 10 and 5 ml. quantities of chloroform, the mixed chloroform solutions being made up to 25 ml., and submitted to a determination of light absorption at 560 mμ with a spectrophotometer. The determination is completed as rapidly as possible to avoid errors due to temperature changes, evaporation and the sensitivity of the colour to light. Beer's law applies at the optimum *p*H, 2·9. Results are compared with those of the titration and the Kjeldahl methods. In the former, the end-point is difficult to determine because of the buffering power of the pilocarpine salts in aqueous solution, and the Kjeldahl method is unreliable since partly decomposed pilocarpine may have the correct nitrogen content. G. B.

Solanaceous Alkaloids, Colorimetric Assays for. A. B. Colby and J. L. Beal. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 351). An examination of two previously described colorimetric assays for solanaceous alkaloids has been made to determine their suitability for routine use. The first was based on the purple colour formed in Vitali's test, the second involved the use of ammonium reineckate to precipitate the alkaloids as reineckates, which were then dissolved in acetone to form a coloured solution. Details of the two assay procedures are given together with the results obtained with stramonium and belladonna. It was concluded that the reineckate assay gave results which were more comparable to the U.S.P. XIV assay than were the results of the Vitali assay; the standard error for the reineckate assay was smaller than for the official assay. The colour developed in the reineckate assay was much more stable than the colour developed in the Vitali assay, although the latter was the more sensitive. The Vitali assay was the more rapid of the two assays studied although both were quicker than the official process and required less material. R. E. S.

Thiourea, Volumetric Estimation of. K. Appa Rao and K. Neelakantam. (*Indian J. Pharm.*, 1952, **14**, 50). The method of Mahr (*Z. Anal. Chem.*, 1939, **117**, 91; 389; *Angew. Chem.*, 1939, **52**, 618) for the estimation of thiourea using bromate was investigated and found to yield erratic results. Slow titrations yielded better results than fast and even then

the errors were of the order of 4 to 8 per cent. on the calculated titre values. The high acid concentration (6·8N) recommended by Mahr in all cases was found to be neither necessary nor desirable. A modified method using potassium permanganate in the place of bromate, and in the presence of potassium iodide as "oxidation buffer" was found to yield better results, the errors ranging from 0 to 1·3 per cent. for amounts of thiourea ranging from 20 to 154 mg. at an initial acid concentration of 6·6N. The results are more easily reproduced and the method is recommended for general use. R. E. S.

Tocopherol, Colorimetric Determination of. C. Domart. (*Ann. pharm. franç.*, 1952, **10**, 199.) The determination depends upon the oxidation of tocopherol in ethanolic solution by ferric chloride. The quantity of ferrous ions produced is estimated from measurements of the stable red colour formed by reaction with $\alpha:\alpha'$-dipyridyl. The wavelength of maximum absorption is 522 mμ and Beer's law has been found to apply. Ferric chloride also absorbs in the red region of the spectrum, but the excess may be removed by the addition of potassium fluoride which forms a colourless complex provided the *p*H is not less than 3·2. The dipyridyl ferrous colour being stable within the *p*H range 3·5 to 8·5, it is convenient to carry out the determination at about *p*H 4·1. The following procedure is recommended. To an ethanolic solution of tocopherol add 1 ml. of a 0·2 per cent. ethanolic solution of ferric chloride, shake, add 1 ml. of a 0·5 per cent. ethanolic solution of $\alpha:\alpha'$-dipyridyl, shake and allow to stand for 8 to 10 minutes. Add 2 ml. of a 0·5 per cent. ethanolic solution of potassium fluoride, shake, allow to stand for 2 minutes, add 5 ml. of buffer solution at *p*H 4·1 and dilute to 25 ml. with ethanol. Determine the red colour by comparison photoelectrically with a similar solution prepared without tocopherol (Meunier instrument, filter No. 49). The tocopherol content is read from a standard curve. G. B.

GLYCOSIDES, FERMENTS AND CARBOHYDRATES

Cardiac Glycosides, Paper Chromatography of. C. H. Hassall and S. L. Martin. (*J. chem. Soc.*, 1951, 2766.) The procedure is described, and the R_F values are recorded for the cardiac glycosides using a variety of solvent mixtures. The results indicate that all the glycosides investigated may be identified by this procedure when suitable solvents are employed. A. H. B.

GUMS AND RESINS

Resins, Paper Chromatography of. J. S. Mills and A. E. A. Werner. (*Nature, Lond.*, 1952, **169**, 1064.) The work deals with the application of paper partition chromatography to the identification of natural resins and the separation of the constituents, with particular reference to dammar and mastic. The separation can readily be achieved in a reversed-phase system using odourless kerosene (boiling range 170° to 200° C.) as the stationary phase on the filter paper and aqueous *iso*propanol saturated with odourless kerosene as the mobile phase. A descending chromatogram is used and details of technique are given; the strips are dried, developed by spraying with a 50 per cent. w/v solution of phenol in carbon tetrachloride and exposing to bromine vapour for a short period. Resin components which give a positive Halphen-Hicks test show as coloured zones varying from pink to violet. Dammar, mastic, sandarac, rosin, elemi and copal, give characteristic chromatograms which may be distinguished by the number, colour and R_F values of the coloured zones. R. E. S.

ORGANIC CHEMISTRY

Œstrone, Purification of. H. Braunsberg. (*Nature, Lond.*, 1952, **169**, 967.) Traces of an impurity are revealed when the *p*-nitrobenzeneazodimethoxyaniline (fast black salt K) derivative of commercial œstrone is submitted to paper chromatography by the method of Helftmann. The impurity, which yields a blue spot of lower R_F value than the pink dye due to the œstrone derivative, may be equilenin which gives a blue spot of similar R_F value. The impurity may be removed by partition chromatography on celite-sodium hydroxide columns.

G. B.

Visnagan, Crystalline. E. Smith, L. A. Pucci and W. G. Bywater. (*Science*, 1952, **115**, 520.) An optically active extract of the seeds of *Ammi visnaga* L. from which khellin and chellolglycoside had been removed was submitted to chromatographic analysis, using optical activity and ultra-violet absorption as a guide to selecting eluted fractions. An amorphous product, $[\alpha]_D + 16°$ was re-chromatographed and crystalline visnagan, obtained from a central fraction by crystallising from methanol at 4° C. on prolonged standing, and recrystallised, had the following characteristics:—m.pt., 86° to 88° C., $[\alpha]_D + 12{\cdot}5°$, molecular weight, 373. Its vasodilatatory effect in isolated rabbit hearts was 8 times as great as that of khellin. A further chromatographic fraction, $[\alpha]_D + 50°$, yielded a crystalline compound of m.pt. 157° to 159° C., $[\alpha]_D + 96°$, and molecular weight 276. The ultra-violet absorption spectrum closely resembled that of dihydrokhellin. This substance appeared to be a dihydrofuranochromone. In isolated rabbit hearts this substance was as potent a vasodilator as khellin.

G. B.

BIOCHEMISTRY

GENERAL BIOCHEMISTRY

Noradrenaline and Accessory Chromaffin Tissue. D. M. Shepherd and G. B. West. (*Nature, Lond.*, 1952, **170**, 42.) Following the finding that the collection of chromaffin tissue in babies, known as the organs of Zuckerkandl, contains large amounts of noradrenaline, experiments were conducted (*a*) to confirm that a sympthomimetic substance is present in accessory chromaffin tissue of animals, and (*b*) to identify such a substance by biological and chromatographic methods. The results indicated that large amounts of noradrenaline may be found in the retro-peritoneal tissue of young dogs rabbits, guinea-pigs and cats, exceeding in some cases the amount found in the suprarenal glands. These additional chromaffin structures must perform some autonomic function early in life, possibly the maintenance of blood pressure. As the animal grows older and the suprarenal medulla matures, so the amine content of this accessory tissue declines. Extracts of the carotid bodies and of the aortic bodies of rabbits, cats and guinea-pigs did not yield any sympathomimetic material; this may be explained by the fact that chromaffin tissue is not necessary for the production of adrenaline and noradrenaline. Precursors of noradrenaline, such as hydroxytyramine and dihydroxyphenylalanine were not detected in any of the extracts.

S. L. W.

BIOCHEMICAL ANALYSIS

Dried Plasma, Water Content of. H. Sager. (*Pharm. Acta Helvet.*, 1952, **27**, 121.) The activity of a comparatively stable product such as diphtheria anti-toxin decreases much more rapidly in the form of a solid containing 5 to

8 per cent. of water than when in solution. Thus the water content of such materials is of great importance for stability. It has been shown that under tropical conditions large quantities of water are able to penetrate through rubber bungs. When removing water by freezing, 3 factors are important in order to attain a low final water content: the thickness of layer of the frozen product must be as uniform as possible; the cooling at the beginning of drying must be sufficient to avoid water inclusions; and de-mixing in the form of separation of pure ice on the walls of the container must be avoided, as this prevents the direct contact of the product with the walls. The minimum residual moisture content depends on the temperature of the condensing surface, the maximum permissible temperature for the product, and the vapour pressure curve of the product. For the determination of water content, 3 methods were compared—the U.S.P. method (phosphorus pentoxide), the Thomann and Kaelin method (distillation with xylene) and the Karl Fischer method. The results obtained diverged considerably. Moreover all these methods have the disadvantage that the contents of the container, after removal of the sample, cannot be used for transfusion. The author is attempting to develop a new method based on the measurement of vapour pressure, which rises very steeply with increase in water content. The starting point is a "physically dry" plasma prepared at a plasma temperature of + 50° C., using a condenser cooled with liquid air. Results of these experiments are to be reported later. G. M.

Hydrazine Derivatives of *iso*Nicotinic Acid in Blood, Determination of. S. H. Rubin, L. Drekter, J. Scheiner and E. de Ritter. (*Dis. Chest.*, 1952, **21**, 439). A colorimetric method is described for the determination of blood plasma levels of *iso*nicotinic acid and its derivatives, the hydrazide and the *iso*propylhydrazide. The substance is converted to *iso*nicotinic acid and determined colorimetrically by the reaction with 10 per cent. cyanogen bromide and ammonia described for nicotinic acid (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 513); the hydrazide derivatives, which yield relatively slight colour *per se*, are converted to *iso*nicotinic acid by treatment of the protein-free plasma with permanganate. A blank determination to correct for non-specific colour can be made by assay of a control sample taken before dosage or the blank can be estimated by the reaction with 3 per cent. cyanogen bromide and metol, in which *iso*nicotinic acid yields no colour. Details of the procedure are given. Results are given of plasma levels over a 24-hour period after a single oral dose for dogs having 3·5, 7, or 14 mg./kg. and for humans receiving up to 3·4 mg./kg. of *iso*nicotinyl hydrazide and *iso*propylhydrazide. Maximum plasma levels were found within one-half to 2 hours after the dose and declined rapidly. No significant amount of either compound was found in the plasma after 24 hours. R. E. S.

***iso*Nicotinyl Hydrazide in Biological Fluids, Estimation of.** J. M. Kelly and R. B. Poet. (*Amer. Rev. Tuberc.*, 1952, **65**, 484). Two methods are described for the estimation of *iso*nicotinyl hydrazide in blood plasma or urine. The substance is extracted from alkalinised plasma or urine into an *iso*amyl alcohol-ether-ammonium sulphate system and subsequently estimated either spectrophotometrically or colorimetrically after extraction into 0·1N hydrochloric acid. The spectrophotometric method using the absorption peak in the ultra-violet at 266 mμ is reliable for concentrations of 5 μg./ml. in

plasma and 20 μg./ml. in urine. The colorimetric method using *p*-dimethylaminobenzaldehyde as the reagent has a lower limit in plasma of 1 μg./ml. and 5 μg./ml. in urine. Details of procedure are given; blank determinations on the biological material are necessary. R. E. S.

Penicillin in Culture Fluids, Determination of. A. Beloff-Chain and F. Dentice D'Accadia. (*Analyst*, 1952, **77**, 423). A rapid iodimetric method of determining penicillin in culture fluids or crude preparations is described. The penicillin is extracted from an aqueous phase with amyl acetate at *p*H 2 and is re-extracted from an aliquot of the amyl acetate solution by a known volume of phosphate buffer at *p*H 7. The penicillin is then assayed by the iodimetric titration method, details of which are given. A blank determination must be made to correct for substances other than penicillin present in the culture fluid that are extracted and that take up iodine under the specified conditions; in addition some of the inactivation products of penicillin are extracted by amyl acetate and take up iodine, and a correction factor depending on the penicillin present in the original broth must be subtracted from the titration value of the blank. Results on culture fluids are given and it is concluded that for all normal routine penicillin fermentation work the rapid assay is sufficiently accurate between the limits of 150 and 1200 I.U./ml.; at concentrations of less than 150 I.U./ml. it is advisable to use the biological assay method; above 1200 I.U./ml. it is necessary to dilute the culture fluid before assay. R. E. S.

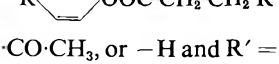
Urinary 17-Ketosteroids, Analysis of. B. M. Bray. (*Analyst*, 1952, **77**, 426). An examination of the methods available for the preparation of crude urine extracts for 17-ketosteroid analysis has been made. 3 methods for isolating the ketonic fraction from a mixture of urinary steroids by means of Girard's reagents were compared with respect to reproducibility, the time required for the formation of the hydrazone and the degree of dryness necessary in the reaction mixture. Condensation with Girard's reagent T for 3 minutes in a bath of boiling water gave an average recovery of 95 per cent. for amounts of dehydro-*iso*androsterone between 0·5 and 2·5 mg. and 94 per cent. for the same steroid added to a urinary extract. Girard's reagent P gave lower results. Drying the urinary extract for 12 to 36 hours *in vacuo* did not improve the recovery. The method of Talbot *et al* (*J. biol. Chem.*, 1940, **132**, 595) was used for the hydrolysis of the hydrazone; for the estimation of the sterone Zimmermann's reaction was used. R. E. S.

Vitamin A, Reproducibility of Geometrical Correction Procedures in the Spectrotrophotometric Estimation of. H. H. Bagnall and F. G. Stock. (*Analyst*, 1952, **77**, 356). Recent assessments of the precision of geometrical correction procedures for the spectrophotometric estimation of vitamin A are discussed. Results are given for correction procedures as applied to halibut liver oils; for the estimation of vitamin A, the method of the B.P. Addendum 1951 was followed except that all three correction equations recommended by Cama (*Biochem. J.*, 1951, **50**, 48) were used with each of three separate weights of oil dissolved in *cyclo*hexane so giving 9 "corrected" values for $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 327·5 to 328 mμ. The fiducial limits (P = 0·05) of the mean of the 9 values from $\pm$ 0·60 to $\pm$ 2·33, with average value $\pm$ 1·17 (intra-laboratory). Results were then calculated. The P = 0·05 fiducial limits of the mean of 9 values for the corrected $E_{1\text{ cm.}}^{1\text{ per cent.}}$ at 328 mμ, expressed as a percentage of the mean, varies are also given of a small scale inter-laboratory test. R. E. S.

CHEMOTHERAPY

Diphenylmethane and Diphenylamine, Some Alkylamino Derivatives of. G. Benoit R. Delavigne and F. Elipoulo. (*Ann. pharm. franç.*, 1952, **10**, 181.) Diphenylmethane derivatives of the general formula $(C_6H_5)_2{\cdot}HC{\cdot}(CH_2)_n{\cdot}N(C_2H_5)_2$, HCl were prepared where $n = 1$ to 6 and 10. Compound $n = 1$ was prepared by catalytic reduction of diphenylacetonitrile in the presence of Raney nickel, followed by ethylation. Compound $n = 2$ was made by the interaction of diethylaminochloroethane and the sodium derivative of diphenylmethane, and a similar process was applied for compound $n = 3$. Compounds $n = 4$ and 5 could not be obtained in this manner because cyclisation occurred. They were prepared from the phenoxyalkyl bromide by reaction of the magnesium derivative with benzophenone, followed by dehydration of the tertiary alcohol produced and hydrogenation of the double bond. The phenoxy group was removed with hydrogen bromide and the product heated in a sealed tube with diethylamine. A corresponding series of derivatives of diphenylamine of the general formula $(C_6H_5)_2\,N{\cdot}(CH_2)_n{\cdot}(C_2H_5)_2$ was prepared. The lower members were obtained by condensation of diphenylamine with the appropriate diethylaminoalkyl chloride in the presence of sodamide. For the higher members of the series the phenoxy derivative was heated with hydrochloric acid in a sealed tube and the product treated with diethylamine. The antiacetylcholinic and antihistaminic activities of the diphenylamine derivatives were much less than that of the corresponding members of the diphenylmethane series. G. B.

Phenyl Esters of β-Dialkylaminopropionic Acids as Antispasmodics. T. O. Soine and F. E. DiGangi. (*J. Amer. pharm. Ass., Sci. Ed.*, 1952, **41**, 236.) Derivatives of phenyl propionate of the type



where R = $-NO_2$, $-NH_2$, $-NH{\cdot}CO{\cdot}CH_3$, or $-H$ and R′ = diethylamino, di-*n*-propylamino, di-*n*-butylamino, di-*n*-amylamino, di-*iso*amylamino and piperidino were prepared. *p*-Nitrophenyl β-chloropropionate was made by heating β-chloropropionyl chloride with *p*-nitrophenol and magnesium in benzene, and purified. The β-dialkylamino derivatives were prepared by heating this substance under a reflux condenser with the dialkylamine in ether, filtering, drying the ethereal solution with sodium sulphate, adding a solution of hydrochloric acid in ether and crystallising. Corresponding *p*-amino compounds were prepared by reduction with hydrogen in the presence of palladium on charcoal and ethanolic hydrogen chloride. The *p*-acetylaminophenyl compounds were prepared from *p*-acetylaminophenyl chloropropionate, obtained by catalytic reduction of the nitro compound, and reaction with acetic anhydride. Melting points and analytical data are given. *p*-Aminophenyl β-dialkylaminopropionates showed a moderate antispasmodic activity, and the other compounds are undergoing pharmacological testing. G. B.

Sulphonamides: Relative Potencies and Specificity of. J. Francis. (*Brit. J. Pharmacol.*, 1952, **7**, 189.) The relative potencies of the common sulphonamides and of 4:4′-diaminodiphenyl sulphone were compared *in vitro* against a number of pathogens. Though not a "sulphonamide," the sulphone was included because its action is strongly antagonised by *p*-aminobenzoic acid. The compounds were divided into a non-heterocyclic group (sulphanilamide, sulphaguanidine and sulphone) and a heterocyclic group (sulphapyridine,

sulphamezathine, sulphamerazine, sulphadiazine and sulphathiazole). The sulphone was more active than any other compound against *Str. pneumoniæ* and *Str. pyrogenes*, with the exception of sulphathiazole Against all other organisms (*Past. septica Bact. coli, Salm. cholera suis, N. gonorrhœa, N. meningitidis*) the non-heterocyclic compounds were less active than the heterocyclic. The latter group were nearly always placed in the same order of activity, sulphapyridine being the least potent and sulphathiazole the most potent; the exceptions were sulphamezathine against *Str. pyrogenes* and sulphadiazine against *N. gonorrhœa*. There was no difference in the action of sulphamerazine, sulphadiazine and sulphathiazole against *Bact. coli*, whereas there was a fivefold difference with some other organisms. In the most satisfactory series of experiments the average sulphanilamide coefficient of the heterocyclic sulphonamides was only 2·3 against streptococci but 139 against *Salm. cholera suis* and 36 against *Bact. coli*. S. L. W.

PHARMACY

GALENICAL PHARMACY

Hyoscyamine, Racemisation of, in Preparation of Dry Extract of Belladonna. L-E. Fryklöf. (*Pharm. Acta Helvet.*, 1952, **27**, 175.) The rotation of the alkaloids in belladonna extract may be determined by extracting an alkaline mixture of the extract and kieselguhr with chloroform, and passing the solution through a column of kieselguhr, which absorbs the alkaloids. They can then be extracted with chloroform saturated with ammonia and, after passing through a layer of alumina, the colourless solution can be used for a determination of the optical rotation, while total alkaloids may be determined by titration after removal of the ammonia. Application of this method has shown that, while slow drying of the extract at 100° C. leads to a considerable amount of racemisation, drying at 60° C. for 24 to 48 hours has no such effect, while 8 hours at 80° C. is also permissible. In this respect there is practically no difference between an extract containing chlorophyll and one freed from it.

G. M.

NOTES AND FORMULÆ

Piperazine Œstrone Sulphate (Sulestrex Piperazine). (*New and Non-official Remedies, J. Amer. med. Ass.*, 1952, **149**, 443.) Piperazine œstrone sulphate is an equimolecular compound of piperazine and œstrone sulphate stabilised by the addition of a small amount of free piperazine. It is a fine, white to creamy-white, odourless, crystalline powder, slightly soluble in water and ethanol. It melts between 185° and 195° C. to a light brown syrup, which solidifies on further heating and finally melts at about 240° to 250° C., with decomposition. When dissolved in water and boiled with a solution of quinone in ethanolic acetic acid, a currant-red colour appears. When heated with a solution of β-naphthol in sulphuric acid, cooled, and diluted with water, an orange-yellow colour appears which becomes red-orange on further heating. When dried at 105° C. for one hour, it loses not more than 1·0 per cent. of its weight; it yields not more than 0·1 per cent. of sulphated ash. It contains œstrone 56·0 to 63·0 per cent., equivalent to 90·4 to 101·7 per cent. of piperazine œstrone sulphate, and nitrogen 6·4 to 7·0 per cent. The œstrone is assayed by heating an aqueous solution with hydrochloric acid, extracting with benzene,

treating the residue from the benzene solution with a solution of phenol in sulphuric acid, and determining the optical absorption at 5220 Å of this solution and of solutions prepared similarly from standard œstrone. The nitrogen is assayed by a semi-micro Kjeldahl method. G. R. K.

PHARMACOGNOSY

***Colchicum autumnale*, Alkaloidal Content of.** F. Šantavý and T. Reichstein. (*Pharm. Acta Helvet.*, 1952, **27**, 71.) In the spring, when the first leaves are showing, old corms of *Colchicum autumnale* contain 3 times as much total alkaloid as in autumn, and 14 days later the amount is still greater. The largest amount is of substance F, followed by colchicine and finally substance G. Some months later the content in the old corms is reduced, as they atrophy considerably and the new ones grow: only small amounts of colchicine-like substances could be isolated. At the time of ripening of the seeds the old corms were so much atrophied that they could not be analysed; the young ones contained the same substances as later, in the autumn. Small amounts of another compound—substance S were found. Ether-soluble alkaloids were present especially in old corms in the spring months. The results show that in Central Europe the harvesting is most profitable during flowering in autumn: although there is a greater percentage of alkaloids in old corms, in spring, they are more difficult to find, and moreover the corms are larger in autumn and the total amount of alkaloid is greater. G. M.

Digitalis, Occurrence of Free Genins in. F. Neuwald. (*Arch. Pharm. Berl.*, 1952, **285**, 22.) Although free genins are often stated to be present in digitalis leaf, the evidence for this is not decisive, and there are no enzymes in the leaf capable of splitting the glycosides of the digitoxin stage into genins and digitoxose. In order to decide this question the genin-glycoside fraction (free from digitoxose) was extracted, and assayed by the modified genin method of the author (depending on the genin content) and by the digitoxose method of Soos. Tests were carried out on 3 samples of the drug which had been stored under different conditions and contained about 10 per cent. of water. The agreement between the 2 methods was very good and indicates that free genins were absent in the 3 samples, within the limits of accuracy of the photometric determination ($\pm$ 2 per cent.). G. M.

Ergot, Alkaloidal Content of Naturally Occurring. L. Fuchs. (*Scientia Pharm.*, 1952, **20**, 1.) Samples of natural (wild) ergot were assayed by the method of Fuchs for water-soluble and insoluble alkaloids. Most of the 67 samples contained less than 100 mg. per cent. of insoluble alkaloids. Some samples from localities where artificial inoculation had been carried out in the same season showed a considerably higher content (up to 252 mg. per cent.) but this appeared to be a secondary infection, since elsewhere in the same region ergot was found which was almost alkaloid-free. There also appear to be other strains of ergot which are distinguished from one another by the content of water-soluble alkaloids. Thus all samples from Lower Austria showed less than 20 mg. per cent. (generally less than 10 mg. per cent.), while most of those from Steiermark contained more than 20 mg. per cent. and up to 39·5 mg. per cent. of water-soluble alkaloids. G. M.

PHARMACOLOGY AND THERAPEUTICS

p-Aminosalicylic Acid, Effect on the Allergic Process. E. R. Trehewie. (*Med. J. Aust.*, 1952, **1**, 638.) Sodium salicylate and acetylsalicylic acid have been previously found by the author to inhibit the release of histamine from the lungs of sensitised guinea-pigs by the sensitising antigen, egg albumen, and experiments were made to discover whether *p*-aminosalicylic acid also had an anti-allergic effect. Guinea-pigs were sensitised with crystalline egg albumen, and the antigen was injected immediately after being dissolved in Tyrode solution, containing the salicylate compound, in the same concentration as in the perfusing fluid. Samples of the perfusion fluid were collected 5 minutes before and 0 to 10 and 10 to 15 minutes after intra-arterial injection of 5 mg. of the egg albumen. The samples were immediately boiled and the histamine determined on the isolated jejunum of the guinea-pig. The results are tabulated and show that *p*-aminosalicylic acid is a powerful inhibitor of histamine release. The author suggests that the anti-allergic activity of *p*-aminosalicylic acid may be of therapeutic importance in tuberculosis. Since completion of this work evidence has been published that in some tuberculous patients antihistaminics cause an extremely rapid and unexpected subsidence of the pathological process while histamine stimulates the growth of tubercle cultures. It is suggested that *p*-aminosalicylic acid may be of value in certain allergies in which antihistamines fail because inhibition of the antigen-antibody response might be expected to block a wider variety of effects. H. T. B.

Basic Ketones and Related Compounds, Pharmacology of. P. B. Marshall, N. Ahmad and R. E. Weston. (*Brit. J. Pharmacol.*, 1952, **7**, 85.) The 5 series of compounds investigated included: β-dialkylaminoketones, bispidines, γ-dialkylaminoketones, γ-dialkylaminobutyramidines and 2-(γ-dialkylaminopropyl)dihydroglyoxalines. Most are spasmolytics having non-specific direct action on plain muscle. 2 compounds $(C_6H_5)_2CH{\cdot}CO{\cdot}CH_2CH_2NR_2$, where $R = CH_3$ and C_2H_5, possessed fairly high activity. Many of the compounds have well-marked local anæsthetic action. Intracutaneously in guinea-pigs, 10 compounds showed greater activity than procaine while two of the β-dialkylaminoketone series proved to be half as active as cinchocaine. One compound, in the bispidine series, containing a β-chloroethyl group attached to nitrogen, showed some protective action against lethal doses of adrenaline in mice. Its potency is about 1/16th that of dibenamine. None of the compounds was found to have any analgesic or neuromuscular blocking action or very marked action on blood pressure, spleen volume and respiration. J. R. F.

Chloramphenicol Administration, Fatal Aplastic Anæmia Following. L. A. Hawkins and H. Lederer (*Brit. med. J.*, 1952, **2**, 423), B. Wolman (*Brit. med. J.*, 1952, **2**, 426), R. K. Smiley, G. E. Cartwright and M. M. Wintrobe (*J. Amer. med. Ass.*, 1952, **149**, 914), and P. Sturgeon (*J. Amer. med. Ass.*, 1952, **149**, 918). 1 adult and 8 child cases of fatal aplastic anæmia following chloramphenicol therapy are described in the 4 papers. Wolman describes 1, Smiley *et al.* 2, Hawkins and Lederer 2, and Sturgeon 4 cases. The British papers report prolonged treatment with large doses (44 days, 28 g. Hawkins and Lederer), the American papers report intermittent and prolonged treatment. Sturgeon reports 1 case where another drug, an antihistaminic, which has been known to produce the disease, was administered for 1 day only, and 2 cases where exposure to organic solvents and dyes occurred. The other authors

state that no drug, known to produce the disease, was given during the antibiotic treatment. Hawkins and Lederer administered adrenocorticotrophic hormone to both of their patients, 1 and 3 days before death. The hormone had no apparent effect, but no conclusions are drawn, as the period of administration was too short. Wolman and the American authors suggest that as the drug has not produced the anæmia in a large number of cases a small number of persons must possess an idiosyncrasy to it. Smiley *et al.* report that both cases treated by them had a history of allergic manifestations. All the authors conclude that as no other drug with known aplastic anæmia-producing properties had been used during treatment, and as chloramphenicol was the common factor, its nitrobenzene radical, which is a known hæmopoietic toxin, is responsible for the disease. Prolonged treatment is not advised and the possibility of idiosyncrasy should be borne in mind. J. R. F.

Choline Group, Studies of the Structure-action of Related Compounds. H. R. Ing, P. Kordik and D. P. H. Tudor Williams. (*Brit. J. Pharmacol.*, 1952, **7**, 103.) Several groups of substances all related in structure to acetylcholine have been tested on cat blood pressure, rabbit auricles, guinea-pig ileum, frog heart and rectus abdominis. 3 isomeric keto-amyltrimethylammonium iodides exhibted primarily nicotine-like activity, the 4-keto-compound possessing the greatest and the 2-keto-compound the least activity. Both the 4-keto and 3-keto-compounds were equipotent with acetylcholine in producing contracture of the frog's rectus abdominis. The action of the 2-keto-compound was considerably weaker. All 3 produced muscarine-like effects, the 4-keto-compound being consistently the most potent. These effects were largely due to stimulation of ganglion cells since they were considerably reduced by hexamethonium iodide. A comparison of 3 isomeric ethers:—*O*-ethylcholine, *O*-methylhomocholine and *O*-*n*-propylformocholine, showed that the *O*-ethylcholine has the most potent muscarine-like activity and that the activities of the other two compounds were alike and resembled those of *n*-amyl-trimethylammonium more closely than those of choline ethyl-ether. In a furfuryl series, 5-methylfurfuryltrimethylammonium iodide was found considerably more active in its muscarine-like effects than furmethide. Of other compounds investigated, the homologues, acetoxyethyl dimethyl-*n*-propyl- and acetoxyethyldimethyl-*n*-butyl-ammonium iodides, of acetoxyethyldimethylethylammonium iodide, unlike the latter, exhibited only feeble activities and the activities of the sulphur analogue of acetylcholine (acetoxyethyldimethyl-sulphonium) were consistently less than those recorded for acetylphosphocholine. J. R. F.

Dextran in the Treatment of Blood Loss and Shock. J. S. Wilson, E. H. Estes, Jr., J. T. Doyle, W. L. Bloom and J. V. Warren. (*Amer. J. med. Sci.*, 1952, **223**, 364.) The immediate product of the fermentation of sucrose by *Leuconostoc mesenteroides* is a viscous mass consisting of very large dextran molecules. By partial acid hydrolysis the molecules are broken down into smaller units and the authors used a product, fractionated by means of ethanol, in which the average molecular weight was about 70,000, comparable to that of human albumin. It is not known whether the untoward reactions obtained in man with some varieties of dextran are related to the size of the molecules they contain. The product was administered as a 6 per cent. solution in normal saline solution. A preliminary test was carried out on 5 normal healthy men, the systemic and the pulmonary arterial blood pressures, the cardiac output and the blood volume being determined before the experiment, after withdrawal of 450 to 900 ml. of blood, and again after injecting 500 ml. of dextran solution. In

4 cases cardiac output was again determined 45 to 60 minutes later. The bleeding produced a fall in systemic arterial blood pressure only in the subject from whom 900 ml. of blood was taken. No appreciable alteration in cardiac output occurred in any, and all showed a fall in pulmonary arterial pressure and the blood volume. The dextran injection resulted in a return to or above normal in all the factors investigated, apart from the dilution effect. 52 patients with clinical shock were subsequently treated with 500 ml. of the dextran solution. There was a prompt disappearance of clinical signs of shock in 44; of these, 10 subsequently received a blood transfusion and in no instance did the dextran interfere with the typing or cross-matching of the blood. Satisfactory clinical improvement was obtained in all cases with shock due to blood loss, trauma and dehydration. In patients with cerebral trauma or severe infection the results were poor. In general the results were essentially the same as might have been expected from plasma transfusion, and dextran is regarded as a convenient, reaction-free alternative which offers the advantages of large scale production and stability on storage. H. T. B.

Diaminodiphenylsulphone (Dapsone), Treatment of Leprosy with. E. Muir. (*Trans. roy. Soc. trop. Med.*, 1952, **46**, 113.) Diaminodiphenylsulphone is administered orally, starting with about 100 mg. twice a week, gradually increased to 100 mg., 4 times a week. A marked improvement in the bacteriological index of the patients may be observed, especially when treatment commences early. In more advanced cases, especially when reaction-anæmia complex is present, initial dosage must be carefully controlled, and where there is a serious reaction to the drug, adrenocorticotrophic hormone or vitamin B_{12} may be useful for controlling the condition. A lepra reaction, similar to the ordinary lepra reaction which occurs without treatment may be induced by large doses and this is accompanied by rapid destruction of the bacilli and consequent improvement in the bacteriological index. Thiosemicarbazone, streptomycin and chaulmoogra oil or esters may be used in conjunction with sulphone therapy. The effectiveness of diaminodiphenylsulphone in small doses is due to its almost complete absorption when given by mouth, and the slow rate of its excretion. The effect of the substituted derivatives promin, diasone and sulphetrone is due to the diaminodiphenylsulphone which they liberate before or after absorption and consequently they are uneconomical and less certain in action. Treatment in early and moderately advanced cases needs only a minimum of medical supervision, so that the drug could be widely used in endemic areas. G. B.

2:4-Diaminopteridines and Sulphathiazole, Activity of, Against *Streptococcus fæcalis* and *Staphylococcus aureus*. H. O. J. Collier and P. D. Waterhouse. (*Brit. J. Pharmacol.*, 1952, **7**, 161.) The activities *in vitro* of 27 2:4-diamino-pteridines against 4 strains of *Str. fæcalis* were studied. Greatest activity was shown in the dialkyl compounds with straight or branched chains containing 3 to 6 carbon atoms, and also dependent on the substituents being in the 6 and 7 positions. Higher activity was found against strains requiring preformed pteroglutamic acid than against strains able to synthesise this nutrient. The addition of sulphathiazole potentiated the inhibitory effects upon the latter. The inhibitory effect of dibenzylpteridine on *Str. fæcalis* was not antagonised by 5 per cent. human urine. 18 2:4-diaminopteridines were examined *in vitro* against a strain of *Staph. aureus*. The dibenzyl, di*cyclo*hexylmethyl compounds, and the 6:7-dialkyl compounds with unbranched side chains, exhibited greatest activity. The salts of the dibenzyl compound had toxicity similar to

sulphathiazole when administered intraperitoneally to mice. The phosphate was found to act synergistically with sulphathiazole both *in vitro* and in protecting mice against *Staph. aureus* infections. J. R. F.

Hexamethonium and Apresoline, Caution in the Use of. K. S. Grimson, E. S. Orgain, C. R. Rowe and H. A. Sieber. (*J. Amer. med. Ass.*, 1952, **149**, 215.) Hexamethonium (methium) is an orally effective ganglionic blocking agent. Apresoline (1-hydrazinophthalazine hydrochloride) is a weakly adrenolytic and sympatholytic agent with several complex actions. The former may well prove a valuable drug in the treatment of hypertension, while the latter although a less potent antihypertensive agent might aid the treatment of disorders such as toxæmias of pregnancy or early malignant hypertension. Both drugs may produce untoward results and the following precautions should be noted in their use. Therapy should be instituted slowly during observation in a hospital and should be preceeded by thorough physical examination including retinoscopic study to exclude papillœdema with associated encephalopathy and hazard of respiratory arrest from sudden reduction of blood pressure. The heart and kidneys should also be examined since coronory disease makes marked hypotension or tachycardia hazardous, and reduction of pressure can precipitate uræmia. In patients with encephalopathy or damaged myocardium, the drugs should be used cautiously and in small doses. Patients previously treated by sympathectomy usually tolerate smaller doses and should receive very small initial doses. Patients should be warned of untoward effects and told to vary the dose as necessary. Salt-depletion diet with hexamethonium has resulted in low-salt syndrome and barbiturates used with apresoline have caused excessive drowsiness. With either drug, tolerance for ethanol may be decreased. Combined use of hexamethonium and apresoline is under trial, with hexamethonium usually being used first and apresoline being added after a week or more; the procedure is associated with definite hazards. G. R. K.

***iso*Nicotinyl Hydrazide.** R. Knox, K. S. MacLean and J. M. Robson. (*Brit. med. J.*, 1952, **1**, 1081.) The authors give a preliminary account of their two months' experience of the use of *iso*nicotinyl hydrazide in the treatment of human tuberculosis. While confirming that the human tubercle bacillus (strain H37Rv) is sensitive to the drug in concentrations of the order of 1 in 100 million, they find that cultures are not sterilised but become increasingly resistant to increasing concentrations, even without subculturing. A 3 to 4 weeks old culture has shown continuing growth in as much as 8 μg./ml. of the hydrazide. Resistance to as much as 62·5 μg./ml. has been obtained after only three subcultures. This increased tolerance can be delayed or abolished by culturing in the presence of streptomycin. Tests carried out on intracorneal tuberculosis in mice, using bovine and human strains of the infecting organism, showed that if the drug was given in the diet in doses of 12 mg./kg. of body weight per day the infection was apparently controlled completely but if treatment was discontinued after 4 weeks, lesions developed which were indistinguishable from those in untreated animals. Clinical tests on patients with long-standing fibro-caseous disease treated with *iso*nicotinyl hydrazide in comparison with similar patients given lactose showed striking subjective and objective clinical improvement during 6 weeks although the fall in the erythrocyte sedimentation rate was not commensurate with the clinical improvement. The authors suggest that efforts be made to find effective combinations of *iso*nicotinyl hydrazide and other drugs or antibiotics in the hope of delaying the emergence of resistant strains. H. T. B.

Sodium Antimonyltartrate in the Treatment of Schistosomiasis. B. Girgis and A. Magid. (*Trans. R. Soc. trop. Med. Hyg.*, 1952, **46**, 81.) Forty cases of schistosomiasis were treated with varying dosages of sodium antimonyltartrate to determine the optimal dose and duration of treatment. For an intensive course of treatment the optimal dose was found to lie between 12 and 15 mg./kg. of body weight administered in 6 equal injections over a period of 6 successive days. The smaller dose is recommended for patients who may be suffering from moderate degrees of anæmia or other minor ailments. Chronic rheumatic and syphilitic heart disease, emphysema and chronic bronchitis are not absolute contraindications provided the heart lesion is compensated and the injections are given on alternate days while the patient is at rest in bed S. L. W.

Succinylcholine, a Muscle-relaxant of Short Action. J. G. Bourne, H. O. J. Collier and G. F. Somers. (*Lancet*, 1952, **262**, 1225.) The need for a drug capable of giving in the anæsthetised patient a brief but complete muscular relaxation led to extensive tests of succinylcholine. The authors summarise its chemistry and pharmacology and comment on its freedom from toxicity. The drug was administered without misadventure to 546 patients ranging in age from 3 to 86 years, and it was found to be particularly valuable in short procedures such as intubation, electroconvulsive therapy and orthopædic manipulation. It was also used to supplement relaxation when the effects of a long-acting relaxant were waning. Dosage ranged from 5 to 300 mg. in single injections, while up to 2300 mg. was given by intravenous drip continued for 3 hours. In the majority of cases the duration of the relaxant effect of a single dose did not exceed 8 minutes, and in none did it exceed 15 minutes. Details are given of 4 different ways in which succinylcholine was applied to abdominal surgery, namely as a supplement to curare or gallamine, as a supplement to decamethonium in some old and bronchitic patients, as the sole relaxant administered by frequently repeated injections and as the sole relaxant by continuous intravenous infusion. The drug differs from decamethonium in being destroyed by cholinesterases; neostigmine and other anticholinesterases therefore prolong its action. Although used as an antidote to *d*-tubocurarine and gallamine, neostigmine would be worse than useless for succinylcholine. H. T. B.

Succinylcholine Chloride; Self-Experiments. O. K. Mayrhofer. (*Brit. med. J.*, 1952, **1**, 1332.) Self-experiments were carried out by the author to prove the specific action of succinylcholine chloride on the skeletal muscle of man, its safety as a curarising agent for use in anæsthesia, and its freedom from undesirable side effects. The substance was used in the form of 5-ml. ampoules containing 20 mg./ml. of the dihydrate. 6 tests, spread over several days, showed that the muscular paralysis produced on intravenous injection was regular, quick in onset and short in duration. In 3 experiments the injection was given quickly (within 2 seconds), the dose varying between 0·125 and 0·375 mg./kg. of body weight. In the next 2 tests the drug was injected slowly (within 2 minutes) in doses of 0·25 and 0·5 mg./kg. Finally a continuous drip was set up of a solution containing 2 mg./ml. in normal saline, in an attempt to maintain a constant degree of relaxation and to regulate the depth of muscular paralysis at will. No medication other than oxygen by face mask was given. The action of succinylcholine chloride is due to the same mechanism as that of decamethonium, namely depolarisation of the end-plate region of the skeletal muscles, so that it is not counteracted by anticholinesterases. The compound is destroyed rapidly in the body and no antidote is considered necessary. The paralysis lasts from 1 to several minutes according to the dose, and full respiratory power is recovered within 3 minutes after the first movement of the

diaphragm, even following heavy dosage. No side or after effects were observed. There were no attributable changes in blood pressure, pulse rate or electrocardiographic tracings. Consciousness was not lost. The small margin between the relaxing and the paralysing dose, and the rather painful muscle-twitching at the onset of its action when large doses are given, makes the drug unsuitable for use with analgesics; it should be used only by anæsthetists in conjunction with an anæsthetic machine. The possible uses are thought to be (1) to provide relaxation for abdominal and thoracic surgery; (2) to facilitate endotracheal intubation; (3) to overcome severe laryngeal spasm; and (4) as an adjuvant in electric convulsion therapy. H. T. B.

Succinylcholine, Sensitivity to. F. T. Evans, P. W. S. Gray, H. Lehmann and E. Silk. (*Lancet*, 1952, **262**, 1229.) The response to succinylcholine usually lasts for from 2 to 4 minutes, but it has been noted that an occasional patient fails to recover fully for much longer periods. Having had 2 patients who did not recover for 20 and 21 minutes respectively, the authors thought this alarming reaction might have been related to low cholinesterase levels in the patients' red cells or serum. A study was therefore made of the enzymic hydrolysis of succinylcholine *in vitro*, and the serum-esterase level was measured in 4 people who showed a normal reaction to the drug, and in the 2 patients whose response was prolonged. Details are given of the methods adopted. Blood contains 2 cholinesterases; "pseudo"-cholinesterase, present in the serum is unspecific for acetylcholine, while there is the true acetylcholinesterase in the red cells. It was found that the "pseudo"-cholinesterase level was very low in the 2 patients reacting abnormally. The *in vitro* tests shows that succinylcholine is not broken down by true acetylcholinesterase but that it is metabolised slowly by the "pseudo"-esterase. It is a competitive inhibitor of acetylcholine hydrolysis by both enzymes. It is suggested that succinylcholine should not be administered to patients likely to have a low serum-esterase level, such as may be found in liver disease or severe anæmia and after poisoning with anti-cholinesterase compounds. H. T. B.

Synthetic Sweetening Agents, Chronic Toxicities of. O. G. Fitzhugh, A. A. Nelson and J. P. Frawley. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 583.) Cyclamate sodium (sodium *cyclo*hexyl sulphamate), P 4000 (1-*n*-propionoxy-2-amino-4-nitrobenzene), dulcin (4-ethoxyphenylurea) and saccharin were given to rats in varying amounts for periods up to 2 years. Saccharin and cyclamate sodium were found to have no pathological effect whatever at dose levels of 1 per cent. or less of the diet and to cause only slight toxic effects at 5 per cent.; at this level cyclamate sodium caused diarrhœa. Although it is only one-eighth as sweet as saccharin, cyclamate sodium appears to be safe for use as a saccharin substitute. Dulcin was toxic to rats at dose levels of 0·1 per cent. and above. Adenomata of the liver occurred in 10 out of 20 animals on 1 per cent. and in a few at lower levels down to 0·1 per cent. of the diet. Splenic enlargement and darkening began at the 0·1 per cent. level and increased proportionally with the dosage. Anæmia and decreased growth rate were present at 1 and 0·5 per cent. Because of the extensive damage observed, dulcin cannot be considered safe for food or drug use, even in small quantities. P 4000 was also toxic to rats at dose levels of 0·1 per cent. and above. An unusual pathological change was the presence of large amounts of a melanin-like pigment in the thyroid; at the 1 per cent. level the thyroids were slightly enlarged and almost black. A somewhat increased incidence of focal nephritis was present at 0·1 per cent. and above. Dosage levels of 1 and 0·5 per cent. decreased growth rate; the 1 per cent. level increased the mortality rate.

Although P 4000 has about 10 times the sweetening power of saccharin, the ratio of toxicities is less. Moreover, P 4000 has an undesirable anæsthetic action. It cannot therefore be considered to be a safe or desirable sweetening agent.

G. R. K.

Terramycin Base, Serum Levels after Oral Administration. R. Mason, P. Kice, E. L. Caffery and M. M. Musselman. (*J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 587.) Three groups of 12 patients were given 1, 2 and 3 g. of terramycin base respectively. The drug was given by mouth and the serum levels of terramycin determined 1, 2, 4, 6 and 24 hours later. Similar tests were then carried out with terramycin hydrochloride on the same patients. The patients were arranged in groups according to age and the gastric juice of each was analysed for free hydrochloric acid and *p*H in the fasting state and for free hydrochloric acid after stimulation by histamine, both before administration of the terramycin and before administration of the hydrochloride. The group receiving 1 g. had an average maximum serum level of 0·7 μg./ml. 4 hours after administration of the base and of 0·9 μg./ml. 4 hours after administration of the hydrochloride. For the group receiving 2 g., the maximum levels were 2·4 μg./ml. 4 hours after administration of the base and 1·8 μg./ml. 4 hours after administration of the hydrochloride. The last group showed a maximum of 3·1 μg./ml. 4 hours after the hydrochloride but a maximum of 2·2 μg./ml. 2 hours after the base. In all groups there was a demonstrable level throughout 24 hours. The data accumulated were not significant in regard to the relation of variations in serum level to differences in age or gastric acidity.

G. R. K.

Terramycin, Value of, in Surgical Infections. F. F. Niedner and H. P. Lange. (*Dtsch. med. Wschr.*, 1952, **77**, 242.) On account of the complex flora of surgical infections, and the varying resistance of the strains, it is necessary to use an antibiotic with the widest possible range of activity, and one to which the smallest number of strains are resistant. It has been claimed that terramycin most nearly fulfils these requirements. A report is given of 74 cases treated in this way. *Bronchopneumonia*: results were good: 1 patient died later from post-operative pneumonia, 33 recovered. *Infections of the gastro-intestinal region*: Results were good when the source had been removed by operation. Terramycin treatment alone did not prevent the occurrence of, or cure existing, abscesses, The use of terramycin greatly reduced the time of treatment. *Urinary infections*: These were rapidly cured, but there were recurrences, when there were mechanical causes for the infection, so that these must be removed if a permanent result is to be attained. In the other cases infections were overcome by operation and terramycin treatment. *Gall bladder infections*: The acute phenomena were rapidly relieved by terramycin, but final cure was only attained after a combination of surgical and medical treatment. *Erysipelas and burns* were rapidly healed. The length of treatment was on the average 4 days, with a maximum of 9 days. When treatment was discontinued too early there were recurrences, so that the cost of treatment is actually increased by an attempt to economise on the costly preparation.

G. M.

3:5:3′-L-Triiodothyronine in Human Plasma. J. Gross and R. Pitt-Rivers. (*Lancet*, 1952, **262**, 439.) Investigations of plasma of patients who have received radioactive iodine showed the presence of an iodine-containing substance which behaves, on two dimensional paper chromotograms and a keiselguhr column, in a manner identical with that of 3:5:3′-L-triiodothyronine. It is shown that this compound is not an artefact of analytical procedure or of the destructive effects of radiation. The amino-acid is concluded to be a normal

constituent of the organic iodine fraction of the plasma since it has been found in the plasmas of both euthyroid and hyperthyroid individuals. J. R. F.

3:5:3′-L-Triiodothyronine in Myxœdema. J. Gross, R. Pitt-Rivers and W. R. Trotter. (*Lancet*, 1952, **262**, 1044.) 3:5:3′-L-Triiodothyronine has been shown to be a normal constituent of human plasma and to be several times as active as L-thyroxine in the prevention of goitre in rats. Two human cases of hypothyroidism were therefore treated with the triiodothyronine administered intramuscularly as a solution in 0·0004N sodium hydroxide, sterilised by heating in an autoclave. The concentration is not stated. Chromatographic examination before and after sterilisation confirmed the absence of thyroxine or other organic iodine compound. 1 patient received an initial dose of 20 μg., followed after an interval of 1 day by 20 μg. daily for 9 days, then 40 μg. daily for 5 days and 80 μg. daily for 13 days. Marked improvement resulted. The basal metabolic rate rose from −40 per cent. to −4 per cent., the plasma cholesterol level fell from 390 mg. to 170 mg. per 100 ml. and there was a loss of 9 lb. in weight. The second patient was treated with a daily dose of 40 μg. rising to 80 μg. and comparable improvement resulted. Treatment of both patients was continued with L-thyroxine orally. Experience in these cases indicates that 80 μg. of triiodothyronine intramuscularly produces in man an effect similar to that obtained from 100 to 300 μg. of thyroxine orally. It is thought possible that triiodothyronine is the compound directly responsible for the peripheral action of the thyroid gland. H. T. B.

3:5:3′-L-Triiodothyronine, Physiological Activity of. J. Gross and R. Pitt-Rivers. (*Lancet*, 1952, **262**, 593.) The synthesis of this compound has recently been reported by these authors and its formation during the iodination of 3:5-diiodothyronine has been described by Roche *et al* (*C.R. Acad. Sci., Paris*, 1952, **232**, 997). This paper describes the biological assay of the compound by its effect in preventing goitre in rats treated with thiouracil. Its activity is shown to be about three times that of L-thyroxine. The role of triiodothyronine in thyroid function is not yet known but the possibility exists that it is the form of the thyroid hormone that is active in the tissues. S. L. W.

Tubocurarine and Strychnine; Action on Spinal Reflex Excitability. C. G. Bernard, D. Taverner and L. Widen. (*Brit. J. Pharmacol.*, 1951, **6**, 551). The effects of *d*-tubocurarine and strychnine on the monosynaptic extensor reflex and the polysynaptic reflex evoked from a cutaneous nerve were studied in decapitated, low spinal, deafferented, and anæsthetised cats. *d*-Tubocurarine did not influence the polysynaptic reflex response in any of the preparations tested, but strychnine increased the amplitude of the response in all types of preparations. In low spinal preparations strychnine produced an increase in the amplitude of the monosynaptic reflex response parallel to its effect on the polysynaptic reflex. When the segment giving rise to the reflex under test is in continuity with the upper part of the spinal cord, strychnine produces a decrease in the amplitude of the monosynaptic reflex response, probably due to inhibition of the reflex by an increased continuous activity from upper segmental levels caused by the strychnine. In the same sort of preparation, if tubocurarine is given beforehand, strychnine augments the monosynaptic extension reflex; this favours the view that tubocurarine blocks structures exerting an inhibitory action on this reflex. At the segmental level of the reflexes tested, tubocurarine produces an increase in the amplitude of the monosynaptic reflex response but has no effect on the polysynaptic reflex, whereas strychnine increases the amplitude of both reflexes in parallel. S. L. W.

LETTER TO THE EDITOR

The Melting Point of Amidone Picrolonate

SIR,—In the monograph on amidone hydrochloride, appearing in the Supplement to the British Pharmaceutical Codex 1949, the picrolonate is prepared for purposes of identification and its melting point given as being about 160° C. *New and Nonofficial Remedies* (1951 Edition) gives, under methadone hydrochloride, the melting point of the picrolonate as 160° to 162° C.

It has been found in our laboratories that amidone sometimes gives a picrolonate of m.pt. 178° to 180° C. when prepared according to the B.P.C. monograph. There is little doubt that the salt exists in two forms of which the higher melting is the more stable for, in our experience, it is very difficult to prepare amidone picrolonate of m.pt. 160° C., after the high melting form has been obtained in a laboratory.

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October 9, 1952.

ABSTRACTS (*continued from page* 1087)

BACTERIOLOGY AND CLINICAL TESTS

Antibiotics, Tests for Sterility. D. Videau. (*Ann. pharm. franç.*, 1952, **10**, 204.) Except in the case of penicillin, for which a suitable inactivating agent is available, elimination of the antibiotic is necessary in testing for sterility. In the following method, aureomycin was separated from micro-organisms by centrifuging and washing. 15 g. of aureomycin was dissolved as completely as possible in 50 ml. of sterile water, any undissolved aureomycin being separated by decantation. 5 ml. of the solution was added to each of 10 centrifuge tubes, each containing 1 ml. of a sterile 3 per cent. suspension of kaolin in water. The tubes were centrifuged for 15 minutes the speed being gradually increased to 5000 to 6000 r.p.m. and maintained for 5 minutes. The supernatant liquid was removed with a pipette and bulb and replaced with sterile water, the centrifuging and washing process being repeated twice and the deposit finally suspended in 1·5 ml. of water. To ensure great dilution of any remaining antibiotic the contents of the centrifuge tubes were used to inoculate large volumes of media-tubes containing 50 ml. of meat-liver broth for ærobic and anærobic culture and flasks containing 100 ml. of Sabouraud-Langeron medium. Tubes and flasks were examined for growth after 5-days' incubation at 37° and 10 days' at 25° C. The method was effective in detecting the presence of bacteria, yeasts and fungi in aureomycin, and could be applied to other antibiotics. G. B.

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