THE ORAL PENICILLINS IN CLINICAL PRACTICE.

A RE-ASSESSMENT

from

THE LITERATURE, LABORATORY AND CLINICAL PRACTICE.

A THESIS

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The regulations of the University of Adelaide for the degree of Doctor of Medicine require:-

(1) A declaration that the thesis is the writer's own composition. This declaration will be found on page 5.

(2) An indication of where the writer considers the thesis to advance medical knowledge or practice. This is listed in the "original work index" in Appendix II on page 184. It includes original work on the serum protein binding of penicillin in Chapter IV, page 70-82, and the application of this work to penicillin assays (page 102) and clinical assessments (page 110). The conclusions and summary concerning oral penicillins available in Australia are found in Chapter IX on page 149-157. They are presented with the hope that they will remove much of the confusion prevailing in the minds of the medical profession in regard to the multiplicity of oral penicillin products.

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INTRODUCTION.

The organic acid benzyl penicillin is established today as the antibiotic of choice in streptococcal, neisserian, clostridial and treponemal infections, even though 23 years have passed since Florey and his co-workers first introduced it to clinical medicine in 1941. In the field of oral therapy the new oral semi-synthetic penicillins are supplementing older forms of oral benzyl penicillin in the treatment and prophylaxis of streptococcal infections and their concomitants, as well as bringing such organisms as antibiotic resistant staphylococci under control. In addition, an oral broad spectrum member is of use in the Gram negative bacillary infections. One problem is that some people show hypersensitivity to the penicillins, but at present this is not a serious obstacle to their general usefulness.

The year 1958 saw the isolation of the penicillin nucleus 6 amino penicillanic acid, and the production of large numbers of new penicillins and their analogues. So many were tested and placed in the hands of the medical profession that much confusion has resulted as to their relative merits. Not only this, but the high pressure tactics of many drug detailers have caused numerous doctors to prescribe the newest penicillin just because it has been

the latest produced and recommended by the pharmaceutical company.

An acceptable choice of oral penicillins cannot just depend upon the detailer's information about limited artificial experiments on volunteers, nor on the results of intelligent guesses so often employed in general practice; it must depend on a correlation of the clinical situation and response observed in practice with the findings of laboratory tests.

Garrod (1960a) has well said that "antibiotics have forced the clinician to think in bacteriological terms, and those who have best adapted their way of thinking have been most successful in the use of these drugs". Bacteriological control at the present time involves the reception of suitable specimens for culture and sensitivity testing, a knowledge of pharmacological data on penicillin absorption, excretion and in vitro antimicrobial activities, and a final interpretation of body fluid assays, correlated with the clinical response. In certain cases today, such as serious infections with resistant staphylococci, bacteriological control has in fact become a logical obligation - the more so because some of the new penicillins show a marked specificity for these resistant organisms.

And yet, reviewing the literature, we find many differences in opinion concerning the penicillins. There is doubt as to the meaning of fairly wide variations in assay levels, disturbing reports of non-absorbers on oral therapy, uncertainty as to the efficacy of intermittent therapy, and some inability to correlate *in vitro* tests with *in vivo* situations. When one considers clinical trials of the various penicillins, the comparisons are difficult to conduct and control adequately, and interpretations may be quite invalid. The efficacy of an antibiotic in some sickness may be quite uncertain, the body defences being often overlooked - although memories of long drawn out serious infections, before the days of antibiotics, leave no real doubt about their efficacy in general.

Rollo and Burley (1962), after comparing three of the oral phenoxypenicillins in volunteers, conclude "the final assessment of the penicillins can be made only after an extended period of use and observation in clinical practice, especially as laboratory methods cannot determine with certainty the influence of such factors in the individual as distribution in the body and inactivation due to serum binding or to breakdown".

In view of this uncertainty I wish to gather the oral penicillins together as one subject and present the following thesis: "A Re-assessment of the Oral Penicillins in Clinical

Practice". This will include a critical review of the literature with the re-testing of many laboratory claims, a checking of the statements of pharmaceutical companies, and a presentation with interpretation of a large number of penicillin assays and therapeutic responses, conducted from clinical cases over a two year period. The final aim is to foster a logical use of laboratory services, and to add further to the knowledge of penicillin therapy.

DECLARATION OF ORIGINALITY.

The work contained in this thesis is original. The reviews, criticisms, presentations and interpretations have been written by me, and are not the result of any other person's work. All the tests and assays performed have been my own, except where some special studies conducted by Dr. J. R. Coulter and myself have been mentioned.

This thesis does not contain material in a form which has been accepted for the award of any other degree or diploma in any University. It contains no material previously published or written by another author except where quotations and references are given.

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I readily admit the difficulty of adding much to the original contributions of the great and pioneering names of the penicillin era, both in England and America, but I trust that my attempt to bring the subject of oral penicillins together in one thesis will help to clear the confusion resulting from the multiplicity of publications and products.

HISTORY OF ORAL PENICILLINS.

CHAPTER I.

THE EARLY ORAL BENZYL PENICILLIN PERIOD.

Discovery of Penicillin.

Antibiosis, the inhibition of the growth of one micro-organism by another, was known at least 40 years before Fleming, in 1929, first discovered the antibacterial effect on staphylococci of a chance penicillium mould . The active substance was named penicillin, and Gram positive organisms in general were found to be susceptible, toxicity to animals very low, and bacteria more affected than tissue cells. Fleming (1929), went on to show the usefulness of a penicillin broth in differential culture media, in the local dressing of septic wounds, and as effective eye drops in a few cases of neonatal conjunctivitis. Clutterbuck et al. (1932), made the first steps in the extraction of crude penicillin into ether, but finding it relatively labile, pursued it no further. Domagk in 1935, showed prontosil to be efficacious in protecting mice against many pathogenic bacteria, but penicillin's systemic capability was not discovered until studies then being pursued on the antibacterial effect of lysozyme led late in 1939 to the further successful testing and extraction of penicillin by the Oxford workers Chain,

Florey and Heatley.

Duodenal Absorption.

Absorption from the gastro-intestinal tract, as well as absorption from subcutaneous tissue and muscle, was investigated and proved first in experimental animals, and then in man (Chain and Sanders, 1940, and Abraham et al., 1941). Acid inactivation of penicillin being known, it was introduced in man by duodenal tube, by-passing the stomach - 16,000 Oxford units of partially purified penicillin being given, equivalent to 400 mgs. of solid substance. Blood and urine assays then showed the presence of some penicillin, suggesting duodenal absorption in man.

About this time a male baby with a persisting staphylococcal urinary infection was cured by 3-hourly 20 mg. oral doses of penicillin with sodium bicarbonate, after duodenal intubation had failed. This led to the investigation of the effect of administering penicillin protected from stomach acid by gelatin capsules with and without salol coating, and the use of antacids. Success was only moderate for it was not realised that the small dose of penicillin (the only dose possible in those days) was the main limiting factor - not acid instability, or poor dissolution of coated capsules, or the already discovered fact that the kidney excreted penicillin very rapidly, necessitating frequent 3-4 hourly dos-

ing. Florey and Florey (1943), reaffirmed the acid destruction of penicillin at various pH levels, and they tested a preparation of 20,000^{u} in gelatin capsules, enterically coated with cellulose acetate phthalate; and these also produced detectable penicillin in blood and urine. Similar findings were reported in America by Rammelkamp and Keefer (1943a), using aqueous solutions of penicillin, and it was about this time that the only reliable blood levels were found to be obtained by intramuscular injections of 15,000^{u} given 3-hourly.

Within 2-3 years more potent products became available, and Penicillium chrysogenum proved to be a more effective penicillin producer than the original Penicillium notatum. Reading the history of penicillin in Florey et al. (1949), one finds that as America developed its production of penicillin to help the war effort, 1943 saw the first isolation of crystalline sodium penicillin, and this led to the realisation that the English and American products were similar but not identical. The American amorphous product, in regard to its principal penicillin content, turned out to be penicillin G, according to the notation being used for the various natural penicillins then being designated, and this was later termed benzyl penicillin. This was to be the product routinely manufactured by 1949 in both England and America.

In 1945 oral doses of commercial crude penicillin increased to 100,000^{u} with a few even at 300,000^{u} - the Oxford or Florey unit now being the international unit. Reasonably consistent inhibitory blood levels were obtainable for the first time, even though there was wide variation in actual levels among individuals, including some who were non-absorbers, and there were also some discrepancies in results from different laboratories. At this stage it was known that 4-5 times the dose of oral penicillin was needed to give levels equivalent to those obtained by the intramuscular route. Peak blood readings for oral medication usually varied between 0.1 - 0.5^{u} per ml., with urine assays recovering 20% of the dose (McDermott et al. 1945 and 1946, Finland et al. 1945, and Seager et al. 1946).

These workers and others next sought to distinguish the best oral vehicle to use. Aluminium hydroxide, buffering agents such as tricitrates and magnesium trisilicate in suspensions or in capsules, various mixtures with glucose, lecithin, cotton-seed oil, or beeswax in capsules, and penicillin powder alone in water or capsules, all gave similar results when tested by blood and urine assays. Plain capsules or aqueous suspensions were found to be the most satisfactory as the oily mixtures gave the lowest readings. Peak blood levels were relatively high and were reached rapidly ($\frac{1}{2}$-1 hour) when the subject was fasting. They fell

quickly (3-4 hours), and food reduced both the levels and the total absorption. This indicated destruction by stomach acid, although achlorhydrics, previously thought to be better absorbers, showed only a few of their number giving significantly better blood levels when tested with the higher doses. Acid stability still appeared important even though food, gastric motility and the size of the oral dose were also now appearing as governing factors, and it was recommended that doses should be taken sometime before meals.

Clinical Trials.

At about this time Finland et al. (1945), Gyorgy et al. (1945), and Bunn et al. (1945), in America, and Bushby and Harkness (1946) in England, reported the earliest small clinical trials of crude oral penicillins, given in varying doses, 3-4 hourly. They obtained good results in gonorrhoeae and a moderate response in lobar pneumonia, finding that 0.03^{u} per ml. of penicillin in blood was an adequate therapeutic level for very sensitive organisms, including Gram positive and negative cocci, Gram positive bacilli and treponemata. Henderson and MacAdam (1946), showed evidence of good absorption in young children, and various types of infection were treated with some satisfactory results. Leinfelder and Paul (1946), published further studies in adults, and warned that

serious infections should still receive parenteral treatment with penicillin, and also emphasised that infections with many of the relatively resistant organisms such as some staphylococci and streptococci, could only be controlled by blood levels higher than those obtainable by oral therapy.

After 1946 the literature was remarkable only for a number of articles giving further proof of primary duodenal absorption (Seeberg et al. 1946, and Stewart and May 1947). First reports were published on the inhibition of renal excretion of penicillin by caronamide (Crosson et al. 1947, Sweet et al. 1948, and Collins et al. 1948), and Keefer (1947) described the clinical use of crystalline sodium and potassium penicillin G, and the less hygroscopic calcium salt. Further observations on the incompleteness of the absorption of oral products was demonstrated by radioactive penicillin studies (Rowland et al. 1948). Cohlan et al. (1948) found more prolonged oral levels in very young infants, and Maliner et al. (1949) mentioned the possible use of lozenges containing 5,000^{u} for streptococcal prophylaxis.

By 1950 better production methods permitted the introduction of tablets of reasonably pure benzyl penicillin, and we find Boger et al. (1950) using 250,000^{u} tablets for the comparison, in the same individual, of different salts of penicillin, some soluble and others insoluble, but no sig-

nificant difference was demonstrated. Kitchen et al. (1950), compared highly compressed tablets of crystalline procaine penicillin G with lightly compressed tablets containing a binder, and found the latter gave the better result. Wright et al. (1953), later confirmed that all tablets needed to disintegrate fairly quickly for reasonable absorption.

The years 1950-51 produced further papers showing that approximately 2-4 times the levels of penicillin could be obtained with oral therapy using the new blocking agent probenecid, serum levels lasting up to about 8 hours (Boger et al. 1950, and Walker and Hunter 1951). The use of probenecid in oral therapy, with its additional toxicity, was not, however, widely accepted, doubtless because of proof of the efficacy of intermittent therapy in infections suitable for oral treatment (Tompsett 1948, Weiss and Steinberg 1949, and Flippin et al. 1951). The latter workers also produced the first controlled clinical trial using patient groups receiving oral and intramuscular penicillin, both being comparable as to age, and type and severity of pneumococcal pneumonia. The trial included standard treatment with broad spectrum antibiotics and an oral penicillin, the latter being given as 200,000^{u} tablets 12 hourly, but no statement is available relating administration to food intake. One assay method was adopted and the index of therapeutic effectiveness was a

drop in fever representing cessation of toxaemia. The trial revealed all the antibiotics to be adequate in their effect upon the course of the infection within 12 hours in the majority of patients, particularly if below 60 years of age.

A few reports of allergic reactions to oral penicillin came from Keefer (1951) and White (1953), who found only a very small percentage of such reactions in a large army group receiving venereal disease prophylaxis. It was already obvious that oral penicillin was not so troublesome in this respect as either parenteral or topical therapy. Babione et al. (1952), studying the clinical outcome of venereal prophylaxis, found that oral penicillin did not consistently protect sailors against syphilis, at least those receiving 250,000^{u} after each exposure. Low dose penicillin therapy is known to be associated with relapses during the treatment of acute gonorrhoea (Report 1961).

Controlled trials continued to be published. Miller et al. (1954) presented results where the siblings of children receiving rheumatic fever prophylaxis were used as controls of the home environment. The test children received soluble penicillin G 200,000^{u} b.i.d. and were followed up with throat cultures and anti-streptolysin O estimations; the control children were similarly studied but received no prophylaxis. As might be expected, the siblings proved to have significantly higher anti-streptolysin O titres and a

greater number of positive throat cultures. Only an occasional breakthrough in the test group underlined the fact that prophylaxis was worthwhile, even though a constant 100% protection was not possible. This publication also pointed out the need for supervision of oral therapy, particularly in controlled trials, and first suggested the 10 day regime for the eradication of haemolytic streptococcal infection of the throat. Wannamaker et al. (1953), had previously indicated that only one mega-unit was needed to eliminate the streptococcal carrier state.

Benzathine Penicillin G.

Returning to the evolution of the oral product, 1951 also saw the development of benzathine penicillin G, an insoluble organic salt. Stollerman and Rusoff (1952), first reported on its long-lasting effectiveness by intramuscular injection in rheumatic fever prophylaxis, while Bayne et al. (1953), made oral tests of this salt which permitted the preparation of the first stable and palatable oral suspension, 300,000^{u} giving slightly higher levels than the equivalent amount of soluble penicillin G. Wright et al. (1953), on the other hand, reported that buffered potassium G tablets gave better levels than benzathine G tablets, both showing slightly prolonged effects when taken after food, and both revealing a few non-absorbers. The streptococcus tube dilu-

tion assay which was used, however, may not have detected some of the lower therapeutic levels, and the tablets varied in disintegration times from 10 minutes to 2 hours, the softer ones being by far the best.

Many reports of quite well controlled trials of both soluble and benzathine oral penicillin then appeared in the literature. These gave apparently satisfactory results in streptococcal infections, even though levels were very variable and initial treatment was often parenteral (Finberg et al. 1953, Cathie and McFarlane 1953, and Welch et al. (1953). Fairbrother and Daber (1954), again found non-absorbers with oral benzathine G suspension, the actual levels varying between 0.03^{u} and 0.25^{u} per ml. for 300,000^{u} doses, most being in the lower range. However, a Staphylococcus aureus assay was used instead of the more sensitive Sarcina lutea method. Mann et al. (1954), finally confirmed the lower levels obtained by oral benzathine penicillin, comparing it with a new soluble suspension of penicillin G in coconut oil.

At this time a scheme of dosage for children, related to body weight, was suggested by Beasley and MacPherson (1954) who recommended 300,000^{u} for 0-25 pounds and 600,000^{u} for 25-40 pounds, and a benzathine product to maintain a level up to 6 hours. Daily doses of 1 gram of sulphonamide were compared for rheumatic fever prophylaxis with a 200,000^{u} benzathine

penicillin, by Markowitz and Hemphill (1954). The penicillin dosage was low and a few recurrences of rheumatic fever were noted in both groups, but penicillin was the drug of choice because of fewer toxic reactions and the lack of any resistant strains of haemolytic streptococci at that time. This was in contrast to experiences with sulphonamides in mass American prophylaxis campaigns during the war.

Further insoluble salts of penicillin G were tested, and Lepper and Spies (1954), found the hydrabamide salt effective, but with possibly a few more allergic reactions. Boger et al. (1954), tested the benethamine salt, and although it appeared adequate the suspension was not as palatable as benzathine. Benzathine in fact went on to become the commonest standard tablet among the insoluble products, even though it was disappointing in regard to expected prolonged blood levels. When the new acid stable penicillin V was discovered a new era began, and the two were widely compared.

CHAPTER II.

THE PENICILLIN V PERIOD.

Penicillin V Production.

Behrens et al. (1948) in the U.S.A., were the first to produce a number of new penicillins containing different substituted acetic acids as side chains. This was accomplished by the metabolic capability of the penicillium mould itself when specific derivatives of acetic acid were added. Brandl et al. (1953) in Austria, reported on the acid stability of the phenoxymethyl sodium member of this new group, and this opened up a field of enquiry concerning its potential value as an oral penicillin, the other biological properties not being strikingly different from benzyl penicillin.

It proved to be easily crystallizable in a stable acid form, and Spitzy (1953), and Schindelmaisser (1954), and others in Europe introduced it for clinical use. Martin et al. (1955) in America, then presented phenoxymethyl penicillin or penicillin V as a successful antibiotic for respiratory tract infections, cellulitis and cholecystitis, and many workers published comparisons of oral penicillin V with penicillin G.

Laboratory Comparisons.

Jones and Finland (1955), reported on G and V given as 200,000^{u} tablets before and after meals, using streptococcal

and staphylococcal serial dilution assays, and they concluded that V produced somewhat higher and more prolonged levels than G, and that V and buffered G were not significantly affected by a meal in contrast to unbuffered G. Wright et al. (1955), tested the anti-microbial activity of V against many staphylococci, finding it just a little more active than G for many of the relatively resistant strains. S. lutea plate assays revealed higher blood levels with V and twice as much recovery from the urine, V averaging 0.5^{u} per ml. at 1 hour and 0.1^{u} at 6 hours, compared with 0.4^{u} and 0.01^{u} for penicillin G. The cumulative urine figures for penicillin V were 20% of the dose, compared with 10% for G. White et al. (1955), found the majority of staphylococci, group A streptococci and pneumococci to be equally sensitive in vitro to V and G.

Putman et al. (1955), then compared tablets, capsules, and an oral suspension of V, with tablets and capsules of potassium penicillin G, and found higher levels of V particularly with the tablets, and an indication that increasing V doses gave proportionately higher blood concentrations. Satisfactory results also came from the use of a suspension of benzathine V, the insoluble salt permitting the first palatable product for children.

Linden et al. (1955), in tests randomised in regard to food, reported that the percentage of patients who maintained levels of 0.03^{u} per ml. or more was higher with penicillin G,

apparently due to the fact that V was irregularly absorbed. Peck and Griffith (1955), however, tested subjects before and after a standard 550 calorie meal and found that V was not materially affected by food in producing an effective penicillaemia of several hours duration.

Clinical Trials.

Clinical tests by Hart et al. (1956) revealed that equivalent doses of oral V and intramuscular G gave acceptable responses in many respiratory cases, and Breese (1955), in a panel discussion, reported a preliminary figure of 90% cover with oral V in the prophylaxis of streptococcal infection in rheumatic fever patients compared with 95% for oral G. Boger, on the same panel, believed that non-absorbers were probably not to be found with G or V, and yet he was disturbed by a few delayed absorption effects with V, particularly in cases of impending shock from peritonitis. Love and Weir (1955), eradicated infection in a whole group of cases with gonococcal urethritis with 125 mgm. of V given 4 hourly for 24 hours, the occasional relapse also being successfully re-treated. In contrast to this, Marmell and Prigot (1955) had many failures with V therapy of gonococcal infections, and it was not finally discarded until Garrod (1960a), pointed out that V was considerably less active than G against Gram negative species including the gonococcus.

Progressive Comparisons.

Further pharmacological aspects of penicillin V were studied by Anderson et al. (1955), who found it to be of low toxicity, a little stronger in its protein binding than G, and inhibited to some extent by probenecid in its renal tubular excretion. Diding and Frisk (1955), showed superior levels were obtainable by V with probenecid, which were still therapeutically effective after 6 hours. Elias and Merrion (1955), checked the acid stability and solubility of V at pH 3 using a potassium salt, and noted that this salt was stable and yet much more soluble than the acid form. Spitzy (1955), concluded that it underwent more inactivation in the tissues of experimental animals than penicillin G. Boger (1955), feared that many of the new side-chain penicillins might be more toxic than the consistently safe penicillin G, but this was fortunately not borne out by later experience, or by animal tests conducted by Glassman et al. (1955).

Bunn (1956), then commended V on the results of his cross-over study of blood levels in some normal young adults, but also felt that V could not be prescribed in doses appreciably less than those commonly recommended for G. Tablets of penicillin V had increased from the American 62.5 mgm. (100,000^{u}) to 125 and then 250 mgm. (400,000^{u}). Rinsler et al. (1956), in volunteer trials with the acid and calcium

salts of V, found blood levels to be once again twice those of G, and maintained for longer periods, 200,000[u] (English 120 mgm.) being given 2 hours after breakfast.

New doubts on oral penicillins in general were brought out at this time by Mohler et al. (1955), who by domiciliary visits found many patients not taking their tablets after the second or third day, even though a 10 day course had been prescribed. Personal experience certainly confirms their observation that 30-50% do not conscientiously take their tablets, and suspicions even of inadequate hospital supervision are often to be entertained. In Mohler's report, there were about 17% of bacterial recurrences with oral G therapy of streptococcal pharyngitis, compared with 5% for a single injection of benzathine G. Goslings et al. (1963) cleared only 70% of positive throat cultures with V compared to 98% with an injection of benzathine G.

A false concept of the range of infections which would respond to oral therapy also seemed to be evident at this period, with reports of such diseases as subacute bacterial endocarditis being treated successfully by penicillin V in large doses, and yet usually with added intramuscular streptomycin (Quinn et al. 1955, and Cox et al. 1956). Oral therapy was, in fact, appropriate only in moderately severe haemolytic streptococcal and staphylococcal infections, except in the case of young children where strict supervision often

made the oral therapy of the serious infections possible.

Many studies were reported in the following years from Europe, including Russia, but on checking the excerpts concerning oral G and V, one does not find anything of added significance. For convenience the literature of the English speaking world will form the bulk of the later information contained in this history and, as before, only those articles which appear to give added information will be quoted.

Tests with penicillin V continued and Heatley (1956), showed a probable linear relationship between penicillin V urinary excretion and serum concentrations, and found that the excretion patterns in three different experiments were not uniform, presumably due to variations in the patterns of stomach emptying and consequent absorption. This implied that urine assays also gave a reasonable measure of the oral absorption of penicillins. Brown et al. (1956) noted considerable variations in assay levels of V, and this precipitated a controversy concerning the relative merits of the acid salt and the new soluble potassium salt. The latter gave higher blood levels in spite of the fact that it was more acid-labile (Juncher and Raaschou 1957, Peck and Griffith 1957, Wright and Welch 1958, and Kraushaar and Giovannini 1958). On the other hand patients with high gastric acidity were found by Spitzy and Doujak (1958), to give better levels after 12 and 13 hours when receiving 250 mgm. 6-hourly of acid V. Berlin and Brante (1958), then tested the acid,

potassium and calcium salts and concluded that the differences were probably without practical importance.

Clinical trials also continued and Wehrle et al. (1956), reported favourably on V in the treatment of scarlet fever, and yet noted that throat cultures did not clear as rapidly on oral as on parenteral penicillin. Finke (1956), used V effectively for the prophylaxis of disabling respiratory infections, and commended it openly because "unlike penicillin G it could be taken independently of meals". McLeod (1957), then said categorically that V was the oral penicillin of choice, defining the ultimate critical test as the successful treatment of some cases of endocarditis. Wehrle et al. (1957) and Fraser (1958), on the other hand, found that a full 8-10 day course of therapy was needed to eradicate streptococcal throat infections both with V and G. Clarke et al. (1957) showed that potassium penicillin G in starch and sucrose tablets was no more affected by food than was V under the same conditions, but a marked drop of V with food, particularly in the absorption of soluble salts, was noted by Juncher and Raaschou (1957).

Further comparisons of oral V and G in the treatment of respiratory infections in general practice were made by Lombardi et al. (1958), with most conditions showing marked improvement, bronchitis being the most tardy in response.

They brought out conflicting views concerning plasma binding in assessing V and G, although Smith et al. (1956), had shown that calculated levels of unbound penicillin for equivalent doses of V and G were very similar in magnitude. Finke (1957), obtained excellent responses in the management of infective asthma in children, using penicillin V in combination with tetracycline. Wheatley (1958), found that 92% of general practitioners used 4-5 day courses of oral V in a 5/2 ratio over parenteral therapy, with 75% stating that it was generally as effective as the parenteral antibiotic, for such conditions as tonsillitis, otitis media, acute bronchitis, pneumonia and minor boils.

One or two papers originating from North America and the continent reported tests on new coated penicillin tablets, the outer coat containing its own portion of the antibiotic. However, no advantages seemed to accrue from their use (Ballon et al. 1958). The year 1957 then saw the patenting of processes for coating tablets with various types of cellulose films, the claim being made that this was a more stable product. At least one firm continued to market penicillin V as a film tablet, but the general stability of compressed tablets is so good today that ease in swallowing is probably the only advantage for the film tablet (Martin and Cook, 1961).

Many other trials were now reported, but Weiss et al. (1959) reviewed the subject and concluded that most penicillin V studies had not been sufficiently controlled. This opinion was supported by the work of Schalet et al. (1958), who showed that there was still no controlled therapeutic evidence that V was more advantageous than G in the treatment of streptococcal pharyngitis. It was, furthermore, necessary to consider the lower cost of penicillin G as compared to penicillin V, and the necessity to buffer the former in the presence of food to obtain serum levels equivalent to those achieved with penicillin V. Other incidental articles showed V to be unreliable in aged people (Goodman 1959). Potassium penicillin V showed enhanced absorption in the presence of added vitamin C (Herold et al. 1959); while penicillin V benzathine and hydrabamine salts in oil or water suspensions gave adequate therapeutic levels (Keen and Mathews, 1960).

Prophylactic Penicillin.

Considering oral benzathine G, Griffith (1958) stated that it was not adequate as a therapeutic drug, because of blood levels which were only therapeutic for about 2 hours, and its usefulness seemed to be mainly in the realm of streptococcal prophylaxis. Miller (1959), however, did not even recommend it for that, preferring buffered G or V tablets.

Feinstein et al. (1959) advocated monthly intramuscular injections of 1.2 million units of benzathine G, in spite of discomfort at the injection site, because this showed only a 7% breakthrough in streptococcal infection compared with 20% for oral G or suphadiazine, and a 0.3% recurrence rate for rheumatic fever compared with 5% for the oral forms of therapy. These figures compare favourably with some others, and yet probably indicate inadequate supervision of oral prescribing, as Miller et al. (1954), recorded only a 6% breakthrough, and Tidwell (1956) a 0.9% figure. Alexson et al. (1960) finally confirmed the oral penicillins, including V, as being adequate in a controlled community programme for rheumatic fever prophylaxis, although Feinstein (1964a) once again found bigger doses of oral penicillin G to give results similar to his 1959 report. Albam et al. (1964) again stressed the superiority of intramuscular penicillin in a large New York survey, and noted that sulphonamide-resistant streptococci were not common in this adolescent group.

We should at this point remember that Sheehan and Henery-Logan (1957) had managed to synthesize penicillin V, although with some difficulty, but it continued to be produced commercially by special fermentation control. When the penicillin nucleus 6 amino penicillanic acid (6APA) was isolated in 1959, the situation had developed that penicillin

V was accepted by many as the drug of choice for oral therapy, with the cheaper soluble or benzathine G as the main streptococcal prophylactic.

CHAPTER III.

THE SEMI-SYNTHETIC PENICILLIN PERIOD.

Phenethicillin.

The isolation and production, at the Beecham Research Laboratories, of the penicillin nucleus, 6 amino penicillanic acid (6APA), resulted in further investigation into the possibilities of synthesising different side chain penicillins superior in absorbability, less affected by the penicillinase formed by resistant staphylococci, and less liable to produce hypersensitivity reactions (Batchelor *et al.* 1959, and Rolinson *et al.* 1960). In 1959, English and American workers isolated a large number of new penicillins, and the Research Division of the Bristol Laboratories in America then described phenoxyethyl penicillin potassium, the BRL 152 of the Beecham Research Laboratories in England.

This antibiotic was acid stable, absorbed readily from the intestinal tract, no more toxic than benzyl penicillin, and it had an antibacterial spectrum almost as good as penicillin V. It was only a little more serum-bound (averaging 42% compared with V 35% and G 28%). Other interesting features were its lessened inactivation by *B. cereus* and staphylococcal penicillinase, in relation to V, and its comparable rate of disappearance from the serum, the latter observation indicating that major differences probably did not exist in

the rates of excretion, distribution and metabolism. Two isomers were also obtained from the synthesised product, each showing less individual activity than the diastereoisomeric mixture (Perron et al., Gourevitch et al. and Pindell et al., 1959).

Further laboratory studies revealed that the absorption of phenoxyethyl penicillin from the intestinal tract was twice that of penicillin V in the first hour, with an equivalently greater excretion in the urine (Morigi et al. 1959). Serum levels in fasting volunteers were reported by Cronk et al. (1959), to be 2.72, 1.74, 0.5 and 0.08 micrograms per ml. at one half, one, two and four hours respectively after a dose of 134 mgm. They also found higher doses to give directly proportional higher levels, particularly if given before meals. In England, Knudsen and Rolinson (1959) studied phenoxyethyl penicillin, or phenoxypropionamide penicillanic acid, and in fasting volunteers the potassium salt again gave levels twice those produced by equivalent doses of penicillin V, and just a little more prolonged after 5 hours. Serum concentrations were also at least equal to those obtained with an equivalent dose of intramuscular crystalline penicillin G.

Clinical results from Morigi et al. (1959), indicated the new phenoxyethyl penicillin to be quite efficient in the treatment of respiratory tract, middle and external ear, and

skin infections. Moreover, very few allergic ortoxic reactions were seen, but it has since been shown that nearly all the penicillins exhibit cross-sensitivity and reactions are more evident now than in the early days of oral therapy. Edgar (1960), conducted a trial in general practice, prescribing phenoxyethyl tablets in all cases where parenteral penicillin would normally have been employed. He used 125 mgm. for patients up to 10 years of age, and 250 mgm. given 4-6 hourly for those who were older. Ninety-three percent. responded adequately, with boils, carbuncles and dental abscesses appearing to recover unusually rapidly, and these findings were taken to confirm the claims of Gourevitch et al. (1959), and Garrod (1960b), that in vitro tests showed the slower penicillinase hydrolysis of phenoxyethyl penicillin (further proof coming from Swallow and Sneath, 1962). No allergies were observed in this series of 68, but one case developed diarrhoea early in therapy. Schofield (1960), then compared phenethicillin (the then approved generic name) with penicillin V in general practice, pairing infections as far as possible in relation to age, sex and clinical features. This author claimed a faster recovery period with phenethicillin in 33 pairs of cases of tonsillitis, bronchitis, pharyngitis, sinusitis, otitis media and furunculosis.

Marshall (1960), on the other hand, had found the assessment of phenethicillin difficult in general practice,

with fairly frequent recurrences or inadequate responses in tonsillitis, and he concluded that at least 5 day's therapy was needed. Bunn and Knight (1960), successfully treated pneumonia with phenethicillin, 250 mgm. t.d.s. between meals, and they found no better response with higher doses. MacKinnon (1960), treated pneumonia and acute bronchitis with 2 mega units of intramuscular benzyl penicillin followed by oral phenethicillin 250 mgm. 6 hourly, most cases leaving no doubt as to cure after 2-3 days. Douthwaite (1960), found streptococcal tonsillitis responding to 250 mgm. 4 hourly in 5 days and also suggested phenethicillin for prophylaxis in spite of its relatively high price.

Numerous other reasonably controlled clinical trials were also reported. Vollum and Juel-Jensen (1960), studied an epidemic of streptococcal pharyngitis in a residential school, and demonstrated a good response with phenethicillin 250 mgm. q.i.d. for 5 days. Morrison (1961), compared phenethicillin and intramuscular benzyl penicillin in a clinical trial of the treatment of acute otitis media, 40 cases being allotted to each group. Phenethicillin 125-500 mgm. by age twice a day before meals for 4-6 days, and 250,000-1,000,000^{u} of penicillin G by injection daily, both gave good clinical responses, with the oral therapy appearing to be more effective in some of the staphylococcal infections. As the staphylococcus was apparently replacing Streptococcus pyogenes and

pneumoniae in primary ear disease, they therefore recommended oral phenethicillin for the therapy of otitis media. Campeau and Lefebvre (1961), then eradicated Streptococcus viridans in 3 cases of subacute bacterial endocarditis by 1 gram doses of phenethicillin administered 2-hourly over 4 weeks. Some nausea and anal pruritus was noticed at serum levels of 10-12 micrograms per ml., but no toxicity was observed in haemopoietic, hepatic or renal studies.

In 1960, conflicting reports on comparative laboratory tests appeared, and Fairbrother and Taylor (1960) again found phenethicillin to give somewhat better serum levels than penicillin V using a tube dilution assay with extra tubes added between the usual increments. McCarthy et al. (1960) claimed, however, that phenethicillin and penicillin V were absorbed in essentially the same amounts in normal people, both before and after food. It was found later that the phenethicillin used in this study was of reduced potency, yet Griffith (1960), reaffirmed McCarthy's work, and incidentally claimed 2-5 times the serum activity of V when tested against oral potassium penicillin G. Both these workers used tube dilution anti-streptococcal and antistaphylococcal serum activity tests, calculating the total activity by measuring the area below the curves of the comparative graphs plotted from the serum titre readings. Cross-over serum level studies using S.lutea plate assays showed some statistical significance favouring

the results obtained with phenethicillin, but they were not felt to be of clinical importance.

McCarthy and Finland (1960) then published an extensive study of the problems encountered with the different activities shown by various penicillins, once again using tube dilution tests, and expressing penicillin activity as units relative to penicillin G, or equivalents relative to 1 microgram of G, and also as weight of penicillin administered. A control intramuscular penicillin G gave the best overall results, and oral serum levels of penicillin V were double those of G with phenethicillin just a little better than V. On the basis of the antistreptococcal tube tests, however, V provided a little greater serum activity than phenethicillin. With regard to urinary excretion, phenethicillin assayed the greatest proportion of the oral products; and the greatest total activity was shown by an experimental phenylmercaptomethyl penicillin, and a long-acting penicillin G tablet with added penicillin in the outer coat. Colville and Quinn (1962), also compared very large 1,250 mgm. doses of V and phenethicillin by serum activity tests and standard plate assays, and found better levels with phenethicillin but equal serum activity.

Penicillinase Resistance.

Confirmation of the relative activities of the oral

penicillins came from Garrod (1960c), who found Gram positive organisms to be definitely more sensitive to G, closely followed by V, and then phenethicillin. Resistant staphylococci were the exception and were more sensitive to the latter drug, but the new penicillinase resistant methicillin (dimethoxyphenyl penicillin) was, however, the drug of choice for resistant staphylococci, even though it was acid labile and a parenteral drug (Douthwaite and Trafford, 1960). Penicillin V and phenethicillin were again clinically compared in a trial for the treatment of otitis media by Handfield-Jones et al. (1962), with a 90% satisfactory response for both. A consideration of all factors at this time leads to the same conclusion as that drawn by Klein and Finland (1963a), who summarised that these two antibiotics were equally active in many infections.

Propicillin.

The next product to appear was phenoxypropyl penicillin or PA248, later called propicillin, and it was described and compared with penicillin V and phenethicillin by Williamson and co-workers from the English branch of Pfizer Ltd. (1961). These workers studied the potassium salt by bacterial sensitivity tests, and by serum assays expressed as weights of the specific penicillins per unit volume, or units of biological activity in relation to penicillin G. Minimum inhibitory concentrations were very similar for V and phenethicillin

with streptococci and staphylococci, while on a weight basis phenoxypropyl penicillin produced serum levels four times as high as V and 20% higher than phenethicillin, over a range of 1-4 hours. The correlation of these tests showed the three oral penicillins to be more or less equally effective against Gram positive bacteria, but this new drug has not been followed up extensively, apart from a few Japanese and European publications. Frisk and Tunerall (1962), and Kanazawa et al. (1963), tested the antibacterial spectra again, and found that penicillin-sensitive staphylococcal and pneumococcal infections responded quite well, in spite of some allergic exanthemata and gastro-intestinal disturbances. A recent therapeutic trial in England showed no significant difference between ordinary doses of oral penicillin G. phenethicillin or phenoxypropyl (Woodyard et al. 1964).

In the latter part of 1961, Doyle and his associates of the Beecham Research Laboratories in England produced BRL 1341 or penbritin, an oral broad spectrum alpha aminobenzyl penicillin. This was followed by oxacillin (BRL 1400), and orbenin (BRL 1621), members of the newly synthesised acid stable and penicillinase resistant isoxazolyl penicillins, which have a bulky 5-membered ring side-chain added to the usual 6-membered benzene ring.

Ampicillin.

Rolinson and Stevens (1961), then published a study on

oral penbritin or ampicillin, and found it bactericidal against a wide range of Gram positive and negative bacteria. The activity against Gram negative bacilli was similar to that of tetracycline and chloramphenicol, and that against pyogenic cocci was only slightly less than penicillin G. It was not stable to penicillinase, but it was stable in acid solution, and its activity was not greatly affected by serum. Brown and Acred (1961) proved it to be non-toxic to experimental animals, and Knudsen et al. (1961) showed it to be well absorbed orally, with fasting serum levels reaching a peak at 2 hours with greater than 2 micrograms per ml. with doses of 250 mgm. 6-hourly. 30% of the dose was excreted in the urine in 6-8 hours, compared with 12% for an equivalent dose of tetracycline. This result was accepted as therapeutic for Gram positive cocci, haemophilae, salmonellae and shigellae, and some strains of Proteus and E. coli. Doses of 500-750 mgm. were later recommended for Gram negative bacillary sepsis other than that occurring in the urinary tract.

Stewart et al. (1961), treated 28 carriers of intestinal pathogens with quite satisfactory results, except for 6 cases infected with S. typhimurium and 2 with E. coli serotypes which were not cleared by the drug. Three transient skin eruptions developed in this series. Staphylococcal resistance was mostly associated with penicillinase production, whereas some Gram negative bacilli were resistant without in-

activating ampicillin. The authors also recorded some very high urine concentrations of the order of 500-4,000 micrograms per ml., which promised to be useful clinically. Trafford et al. (1962), went on to successfully treat 24 out of 27 patients with urinary infections caused by sensitive organisms, some H. influenzae respiratory infections, and one typhoid carrier. Allergic rashes were encountered also in this series. One half gram of ampicillin with probenecid gave doubled serum levels, which remained high after 6 hours, and Klein and Finland (1963a) later pointed out the decreased urinary excretion of ampicillin with probenecid.

Ross et al. (1962), reported from America that ampicillin was moderately well absorbed in children, and readily absorbed after intramuscular injection, with no toxicity being noted in haemograms, urinanalyses and transaminase assays. Barber and Waterworth (1962), confirmed that penicillin G was superior to ampicillin in its antibacterial activity for most Gram positive organisms. Stratford (1962), studied a large number of organisms by disc sensitivity tests and found ampicillin more active against Proteus mirabilis and Streptococcus faecalis than tetracycline and chloramphenicol. 50% of an oral dose was recoverable in the urine, and urinary infections responded well, together with some wound sepsis, but the author doubted the drug's efficacy in chronic respiratory disease. Ayliffe and Pride (1962), on the other hand, found it effect-

ive in the treatment of exacerbations of chronic bronchitis and yet saw no possible difference in parallel trials with demethylchlortetracycline, penicillin V or streptomycin. Millard and Batten (1963), compared ampicillin and tetracycline in a continuous six month trial in chronic bronchitis, using 500 mgm. b.i.d. of each, and found no significant difference in the results. May et al. (1964), have since found 1 gram 6-hourly for 1 week to give fewer relapses in chronic bronchitis than the earlier low doses or broad spectrum antibiotics.

Additional papers were published, including the work of Kennedy et al. (1963) who showed that 2 out of 3 typhoid patients needed a second course of increased oral doses of ampicillin for cure. An appraisal in the Lancet (1963), concluded it to be a disappointing drug in salmonellosis, probably due to the fact that it was excreted too rapidly. Intramuscular ampicillin was suggested as good therapy for Streptococcus faecalis endocarditis and cholecystitis, and in conjunction with the newer penicillinase resistant cloxacillin, reasonable treatment for undifferentiated pneumonias in children. A later article by Burnell (1964), described its use as an oral drug in the treatment of Streptococcus viridans bacterial endocarditis in a child, the patient dying but revealing healed vegetations at post-mortem. Another leading article in the Lancet (1963), noted that it was probably of no value against rickettsiae and viruses, never ef-

fective against pseudomonas organisms, but always active against streptococci and, to some extent, against staphylococci. Some strains of Proteus and E. coli seemed to acquire in vivo resistance, but group D streptococci were found to be quite sensitive and good levels of ampicillin were assayed from bile, suggesting usefulness in cholangitis. Ivler et al. (1963), favourably compared intravenous and intramuscular ampicillin with the usual antibiotics for the treatment of a limited number of bacterial meningitides, but admitted that the incidence of post-meningitic sequelae was not known. Recent English studies by Willcox (1964) indicate the superiority of oral ampicillin in the treatment of gonococcal infections, while Anderson et al. (1964) recommended ampicillin for acute urinary infections, though not for chronic bacteriurias.

Sleet et al. (1964), conducted a double-blind trial of ampicillin compared with chloramphenicol in an outbreak of S. paratyphi infection, and found high daily doses of the former to be a satisfactory alternative to chloramphenicol, especially in the asymptomatic carrier, as mild penicillin reactions were less worrying than the risk of marrow aplasia. Later reports, however, indicate that ampicillin is slower in giving a satisfactory clinical response. Bullock (1963), reported a cure rate of only 2 out of 5 S. typhi carriers, attributing this result to the presence of gall-stones. Using

larger oral doses, Uwaydah and Shamma'a (1964), cured 7 out of 8 uncomplicated typhoid cases proven by blood culture: 1 gram 6-hourly reducing over 2½ weeks was followed, over a further 2½ weeks, by negative faecal cultures. Whitby (1964), then gave 1 gram doses 6-hourly for 28 days and successfully cleared faecal and urinary carriers of S. typhi, noting nc toxic reactions: the exceptions being those with persistent renal involvement not permitting a cure in urinary cases. Previously Tynes and Utz (1962), had recommended cholecystectomy combined with ampicillin for typhoid faecal carriers, and it is interesting to note the observation of Christie (1964), who found persistent "Vi" titres in chronic faecal carriers (some with gall stones) apparently cured with 1 gram oral doses of ampicillin for 3 months and followed for 9 months. Patel (1964), concluded the present picture of ampicillin in the therapy of typhoid as an antibiotic not as effective as chloramphenicol. Christie (1964), then suggested ampicillin in the convalescent period, following other drugs, and even prophylactically for household contacts.

Oxacillin.

Oxacillin (prostaphlin) or 5 methyl 3 phenyl isoxazolyl penicillin, was patented in the United States by Doyle and Nayler (1961), and it was studied both in North America and on the continent before a conference sponsored by Bristol Laboratories in New York first presented the drug. Kirby et

al. (1962), in their paper, found oxacillin to be 5-8 times as active as methicillin against penicillinase producing staphylococci, even though serum binding was much greater. Tube dilution streptococcal assays showed it to be well absorbed, but with greater variability than phenethicillin. One gram fasting doses in volunteers produced an average peak serum level of 9 micrograms per ml. (falling in 3-4 hours), compared with a 6 microgram peak after a meal (falling in 4-5 hours). Average minimum inhibitory concentrations for representative resistant staphylococci were 1 microgram per ml. in broth and 3 micrograms in 50% serum. A number of staphylococcal infections were also successfully treated orally with doses of 1 gram 4-6 hourly with minimal side-effects, except for an occasional skin eruption and some complaints of epigastric pain, tablets having already been replaced by capsules because of the unpleasant taste.

Rutenburg et al. (1962), reported on oral, intramuscular and intravenous oxacillin, and assayed satisfactory levels for all. Klein et al. (1963b), confirmed these findings and the fact that oxacillin was less active than penicillin G against non-penicillinase producing strains, and most of the other Gram positive cocci. An intramuscular injection of 0.5 gram was roughly equivalent to 1 gram of oral medication, and probenecid raised intramuscular $\frac{1}{2}$ gram levels to ordinary 1 gram figures. Rutenburg and Greenberg

(1964), treated serious infections intravenously, following up with intramuscular and oral therapy, and 79% of 302 cases of staphylococcal sepsis of many types responded satisfactorily, except where there was inadequate surgical drainage. A few Gram negative bacillary superinfections in wounds and the respiratory tract were noted, and a few rashes from allergy, but some previously penicillin G sensitive patients did not react to oxacillin. At this time it was thought that oxacillin was the penicillinase resistant drug of choice in America (Klein et al. 1963a), but Hewitt (1963) had drawn attention to the fact that, for the treatment of pyoderma, oxacillin was not excreted in sweat, and subsequent investigations have shown the English product cloxacillin to be superior, with methicillin the drug of choice for parenteral use.

Cloxacillin.

English workers chose and studied BRL 1621, or orbenin, the soluble sodium salt of 5 methyl 3 chlorophenyl isoxazoyl penicillin, later named cloxacillin (Nayler et al. 1962). It revealed itself to be very similar to oxacillin, satisfactory toxicity tests being performed in animals, with quite acceptable inhibitory and bactericidal tests against Gram positive cocci, with the exception of Streptococcus faecalis. More activity than methicillin was exhibited against penicillin resistant staphylococci even though stability to penicil-

linase was slightly less, and penicillinase production could be induced by exposure to the antibiotic. Cloxacillin was definitely more serum-bound than methicillin, and paper chromatography showed 10% of a metabolite in the urine which was, however, closely similar biologically and therefore not expected to seriously invalidate the standard assay. The rare methicillin resistant staphylococci were also noted to be resistant to cloxacillin.

Knudsen et al. (1962) then confirmed many of these properties and showed cloxacillin to be well absorbed orally and by injection, the levels being somewhat better than with oxacillin. Stewart (1962), edited clinical trials from 6 hospitals in England and noted such successful reports as 73 out of 92 resistant staphylococcal infections responding adequately, with only a few needing intramuscular cloxacillin or methicillin. Lowbury and Miller (1962), treated burns infected with Staphylococcus aureus and Streptococcus pyogenes, and proved oral cloxacillin as effective as methicillin, tetracycline or erythromycin in protecting a large percentage of cases. Stratford (1963), was encouraged by the ability of cloxacillin to cure staphylococcal infections of wounds, enteritis, pneumonia and furunculosis, the oral dose being 500 mgm. 6-hourly by capsule. The average serum levels were 6.5 micrograms per ml. at 1 hour, and the minimum inhibitory concentration for 75% of staphylococci was about 0.25 micrograms in nutrient agar, serum at least trip-

ling this figure. Several Gram negative bacillary super-infections in bedsores, urinary tracts and lungs occurred in this series, and it is noted that these could have been due to hospital cross-infection. Intravenous therapy was not tried, as the lack of any ill effects was not proved at that time, but it has since been found to be quite safe.

From the Proceedings of the Third Interscience Conference in America, Bunn and Milicich (1963), reported on the American studies of cloxacillin, and showed it to be more dependable than oxacillin in its oral absorption. In fact, serum levels double those of oxacillin were assayed, including higher figures 4 hours after the dose. Some superinfections occurred possibly due to "circumstances of rapid killing by the drug with inability of the body to repair the anatomical deficit concomitantly". Gravenkemper et al. (1963), also showed that intramuscular cloxacillin gave better and more prolonged levels than intramuscular oxacillin, and that food did not alter the former's superiority even though administration before meals was preferable. Cloxacillin is, however, more toxic to the liver than other penicillins, and this may yet limit its parenteral use in more serious infections.

Phenbenicillin.

Leaving other literature on the isoxazolyl penicillins, Rollo et al. (1962), of the English Distillers Company, des-

cribed phenoxybenzyl penicillin (phenylacetamido penicillanic acid). This publication precipitated further controversy and what has been termed "the battle of the bloods", as this compound was very similar to the other phenoxypenicillins, with possible combined phenoxy and benzyl attributes. Toxicity tests were satisfactory and higher blood levels were achieved with 250 mgm. given as tablets or capsules, the peak readings being 7 micrograms per ml. Urinary excretion was lower than with penicillin V or phenethicillin, with only 24% of an oral dose recoverable, the majority in the first 3 hours. This proved effective excretion and yet left the problem of why such high blood levels did not show equivalent excretion. Following the administration of the single penicillin, paper chromatography of the urine also showed three active components, the main one being phenoxybenzyl penicillin, penicillin V and phenethicillin showing only traces of other active components. Carter et al. (1962), found serum levels much lower when phenoxybenzyl was assayed after a meal, and minimum inhibitory concentrations were intermediate between penicillin G and V. A clinical trial with penicillin sensitive staphylococcal skin infections, plus some cases of streptococcal sore throat and pneumonia produced a good response.

A Brit. med. J. editorial (1962), then discussed the progressive claims of the oral penicillins, noting the dis-

crepancy of phenoxybenzyl penicillin with its low urinary excretion, and concluded by calling for an independent, expert, full scale comparison of the four phenoxypenicillins. Barber and Waterworth (1962), went on to publish comparative activities of many penicillins against numerous pathogens, and they commented that the higher serum levels of the three newest members of the phenoxy group do no more than compensate for the greater activity of penicillin V.

Bond et al. (1963), also took up the editorial challenge by comparing the four phenoxypenicillins in a comprehensive fashion. They stressed the different protein affinities of the various drugs and the different dissociabilities of the serum complexes; previous studies having determined total penicillin in the blood (bound and dissociated) by dissolving standards in media of similar though variable protein affinities. Instead of using bovine albumin or pooled human sera, they employed homologous serum as a standard diluent, and total assays were checked against free penicillin obtained by dialysis, while sensitivities were tested in serum as well as in broth. Against staphylococci and streptococci, penicillin V and phenethicillin were clearly superior to phenoxypropyl and phenoxybenzyl penicillin, comparative serum binding also being 75% for phenethicillin, 80% for phenoxymethyl (V), 89% for phenoxypropyl and 97% for phenoxybenzyl.

This suggested that V and phenethicillin were to be preferred on the basis of available activity at a given time,

apart from any other assessment. Estimation of free antibiotic revealed the highest levels with phenethicillin, followed by V, suggesting better tissue diffusion. Altered though biologically active forms were found in the urine of all those tested, invalidating to some extent the comparisons of the weights of the penicillins excreted in the urine. Bond (1964), then reported some findings of Rolinson and Batchelor concerning metabolites in the blood, both for cloxacillin and phenethicillin, questioning the real validity of existing serum assays.

Miscellaneous papers on oral penicillins include that of Cheng and White (1962), who found that penicillin V absorption could be upset by incidental oral neomycin therapy. Moesmann (1963) reported good levels for oral penicillins in most cases of malabsorption syndrome; and Moffet et al. (1963) claimed that erythromycin estolate gave fewer relapses in streptococcal pharyngitis than penicillin V. Weiss et al. (1964) reported the successful management of non-specific primary pulmonary suppurations (not Gram negative bacillary or staphylococcal), with oral doses of 3 grams a day of potassium penicillin G buffered with calcium carbonate. With regard to prophylaxis, Feinstein (1964b) mentioned trials of intermittent parenteral penicillin therapy augmenting oral prophylaxis, with cases of suspected streptococcal pharyngitis presenting as a coryza-like syndrome.

Nafcillin.

Other synthetic penicillins recently tested have been the phenoxy-isobutyls, ancillin (biphenylyl penicillin or diphenicillin), and nafcillin (ethoxynaphthamido penicillanic acid). The phenoxy-isobutyl penicillins showed no advantages over other compounds (Klein and Finland, 1963b). Ancillin was introduced in 1961 as SKF 12141, and Sabath et al. (1963) found it to be as active as oxacillin but with alimentary absorption markedly depressed by food, to the extent that it has not been marketed in the U.S.A. Nafcillin was produced by Wyeth Laboratories in 1961, and tests showed low levels with oral 1 gram doses, but good 7-8 microgram peak levels with 1 gram intramuscular doses. Minimum inhibitory concentrations for staphylococci varied between .04-2.5 micrograms per ml., with sensitive strains only a little more affected than resistant. Probenecid was found to potentiate blood levels, and it appeared that this penicillin persisted longer in the body than other compounds because little was recovered in the urine (Smith and White 1962, Whitehouse et al. 1962, Klein et al. 1963a, and Lane 1964). Compared with cloxacillin, nafcillin was equally active against resistant staphylococci, and more so against streptococci including pneumococci (Hopper et al. 1963). Apparently successful clinical reports were published for the oral nafcillin treatment of staphylococcal osteomyelitis

(Smith 1963), and controlled double-blind efficacy trials have shown that the parenteral form incorporates much of the efficacy of both penicillin and methicillin (Martin et al. 1963).

Cephalosporins.

Other natural penicillins have been the cephalosporins N and C isolated first in 1954 and 1956 from a Cephalosporium mould. The C product was active against Gram negative bacilli, but it was too difficult to produce until the nucleus 7 amino-cephalosporanic acid (7ACA) was separated. Recent phenylacetyl, thiopheneacetamido and thienylacetamido derivatives showed promise in the broad spectrum field (Chauvette et al. 1962, Barber and Garrod 1963, and Stewart and Holt 1964). The former is called cephalothin, and is typical of the group, being acid stable but poorly absorbed from the gastro-intestinal tract, toxic enough to be withdrawn in some trial patients, and yet not cross-allergenic with other penicillins (Kirby and Bulger, 1964). The second drug is cephaloridine. The Third International Congress on Chemotherapy (1963), found cephalothin to be penicillinase resistant and active against some naturally occurring methicillin resistant staphylococci, but sensitive to a cephalothinase produced by some Gram negative bacilli and staphylococci. Fusidic acid is another product related to the cephalosporins, and it has recently been used orally against

resistant staphylococci, with or without penicillin G or V for synergism, and with some doubts as to whether the emergence of resistance mutants *in vivo* will prove a therapeutic problem (Godtfredsen *et al*. 1962).

Conclusions.

In the present state of our knowledge and in the absence of any real differences in clinical trials, penicillin V and phenethicillin appear to be the oral penicillins of choice in moderately severe streptococcal infections, with the newer penicillins reserved for their specific indications. New penicillins will no doubt continue to be tested, such as dichlorophenyl isoxazolyl and new derivatives of 7ACA for better oral absorption. The long-term prophylaxis of streptococcal infections favours the use of soluble or benzathine penicillin G tablets, as they are the cheapest and most satisfactory oral product, with the alternatives of intramuscular benzathine G, oral V or sulphonamides. A recent conference of the American Heart Association has questioned the need of long-term streptococcal prophylaxis in rheumatic fever. Siegel *et al*. (1966) published evidence that encapsulation of many recent isolates of haemolytic streptococci was minimal, and that the decline in the frequency and the severity of rheumatic fever was probably explained by a decrease in the virulence of group A strains. Others reported on a double-blind trial of oral penicillin G

and a placebo in youths of an average age of 17.5 years. They showed that there was no significant difference in 278 patient-years of experience. The only conclusion that can be drawn at this stage is that younger children should receive prophylaxis at least until they are 17 years of age.

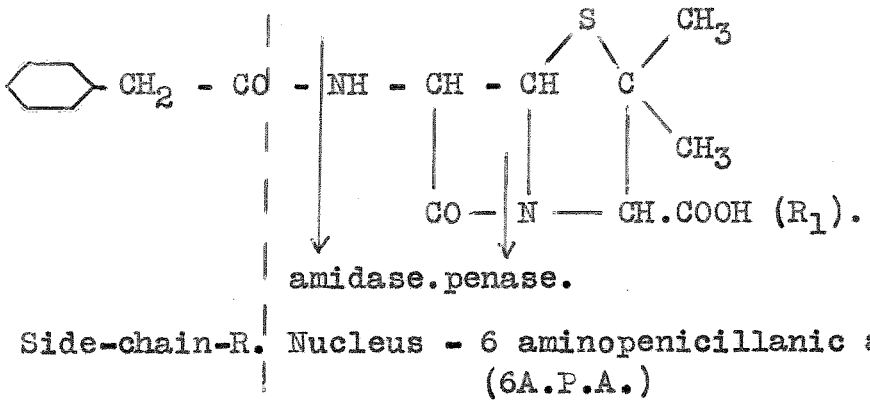
LABORATORY TESTING OF ORAL PENICILLINS.

CHAPTER IV.

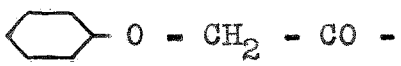
PHARMACOLOGY.

Chemical Structure.

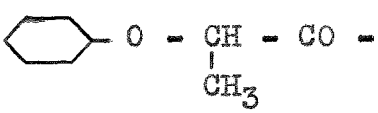
Penicillium chrysogenum fermentation produces benzyl penicillin - penicillin G.



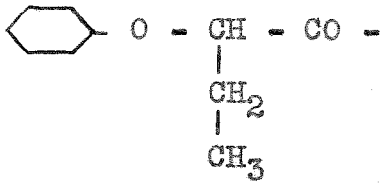
Fermentation with a phenoxyacetamide precursor changes the side-chain to produce phenoxymethyl penicillin - penicillin V.



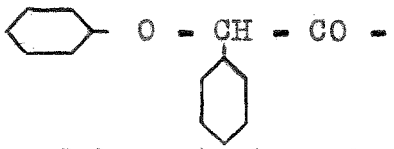
Synthesis of derivatives of separated 6A.P.A. produce other side-chain penicillins, phenoxyethyl penicillin - phenethicillin (a mixture of D and L isomers).



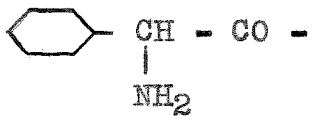
phenoxypropyl penicillin - propicillin.



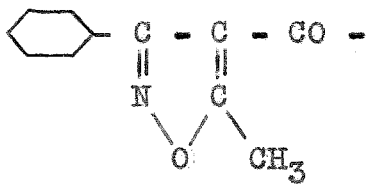
phenoxybenzyl penicillin - phenbenicillin.



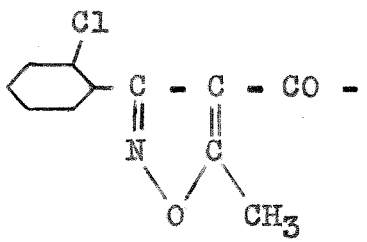
alpha aminobenzyl penicillin - ampicillin (a D isomer).



5 methyl 3 phenyl isoxazolyl penicillin - oxacillin.



5 methyl 3 chlorophenyl isoxazolyl penicillin - cloxacillin.



R_1 represents Na^+, K^+ or benzathine salts etc.

(Behrens et al. 1948, and Hewitt 1963).

Absorption.

All oral penicillins are incompletely and yet rapidly

absorbed from the upper gastro-intestinal tract and particularly the duodenum in man (Abraham et al. 1941, and Stewart et al. 1947). This absorption is somewhat irregular and serum levels can usually be assayed over a 4-6 hour period with peak levels over the first ½ - 1 hour, ampicillin being the exception, producing a peak at 1-2 hours. As the various products and their salts behave differently in the stomach, according to the conditions found at a particular time, and as they must pass through the stomach relatively unharmed and quickly available, acid stabilities including solubilities have been tested, and assessments made of the effects of food on serum levels. Age and weight are not considered to be significant (Bond et al. 1963).

Benzyl penicillin is definitely acid-labile and protecting it from the gastric juices by buffering agents or high dosages given before meals, has resulted in a considerable difference in the absorption of the soluble or crystalline product. The new semi-synthetic penicillins are, however, relatively acid stable and this has resulted in greatly improved absorption (Florey and Florey 1943, McDermott et al. 1945, Brandl et al. 1953, and Gourevitch et al. 1959).

Considering relative solubilities, expected prolonged absorption from insoluble salts (paralleling depot injections) has not been forthcoming, due no doubt to fairly rapid emptying of the normal stomach and duodenum. Insoluble benzathine G has also produced low serum levels with some apparent non-

absorbers, due to the necessity for insoluble penicillin to release acid labile soluble portions before absorption can take place, and to inadequate doses and assay techniques. Losinski and Gleason (1953) commented from their experiments on benzathine G "that the complex penicillin compound undergoes rapid hydrolysis in normal gastric juice ... considerable variation might therefore be found in not only the release of the soluble penicillin ... but also in its subsequent absorption". Later Austrian work showed that benzathine V was rapidly hydrolysed to acid-stable penicillin V acid by the gastric juices, this in turn being converted to soluble V in the alkaline duodenum. The different salts of the semi-synthetic penicillins vary markedly in their solubilities, such as highly soluble penicillin V potassium and relatively insoluble V acid, but this has not greatly affected absorption levels, except to the extent that all soluble salts show higher and more rapid peak serum levels under fasting conditions (Fairbrother and Daber 1954, Wright and Welch 1958).

Table I on page 57 compares measurements of acid stability and solubility found in the literature covering the currently available products.

Table I. Published Penicillin Acid Stabilities of Solubilized Portions.* Expressed as Percentage Loss of Potency over 6 hours at 25°C. and Percentage Solubilities in Various Buffers and Water at 25°C.

	Acid Stabilities			Solubilities		
	pH2	pH3	pH6	pH2	pH6	Water
Penicillin G potassium	100	88	0	-	100	100
G benzathine	100	100	0	0.4	0.6	0.031
Penicillin V acid	25	14	0	1.3	24	.09
V potassium	0	6	4	10	100	100
V benzathine	36	14	9	6.2	3.4	.032
Phenethicillin (potassium)	36	17	-	-	100	-
Ampicillin (acid)	20	-	-	-	10	-
Cloxacillin (sodium)	25	14	-	-	100	-

* (Elias and Merrion 1955, Weiss et al. 1957, Gourevitch et al. 1959, Rolinson and Stevens 1961, and Knudsen et al. 1962).

Food in the stomach is associated with quite variable absorption, and serum levels of penicillin G are lower and less prolonged than when administered to the fasting subject; whereas buffered G gives reasonable levels with or without food. The semi-synthetic penicillin levels, on the other hand, are prolonged by food for upwards of one hour, giving somewhat lower levels, and yet they often exhibit an increase in total absorption, leaving the relationship of dose to food as a relatively unimportant factor. The exception is cloxacillin, as its serum levels and *in vitro* sensitivities are not widely separated.

All this is reasonably explained on the basis of total acidity increasing in the stomach following a meal, the relative acid stabilities of the penicillins and a certain amount of food adsorption. Different absorption and urinary excretion patterns are also adequately explained by different stomach emptying times, with pyloric delay being the reason for the occasional "late" absorbers (Jones and Finland 1955, Peck and Griffith 1955, Heatley 1956). Increasing the length of the molecular side-chains attached to the penicillin nucleus has for some reason, definitely improved the absorption of successive penicillins, but at the expense of antibacterial activity, leaving the acid stabilities fairly constant (Frisk and Tunerall 1963).

Table II, page 60, shows the average peak absorption figures of many previously published serum assays. These were obtained from volunteer oral penicillin experiments. Fasting levels are shown to be always higher than those after food, and the dose-response will generally increase in direct proportion to the dose. Figure 1, page 61, presents average blood level curves from the same sources and this reveals the 4-5 hour limit of assayable oral penicillin. The penicillin G is marketed in units but is presented in this figure as comparative micrograms.

Excretion.

Early workers have used experimental animals in excretion studies on the various penicillins, but crude penicillin was found to be excreted in man via the urinary tract by Abraham et al. (1941). Proof that the kidneys played the largest part in the rapid removal of penicillin from the body came from Rammelkamp and Keefer (1943a), who detected persistent serum levels for many hours in patients with poor renal function, normal levels only lasting approximately 2-4 hours.

Rantz and Kirby (1944) directly correlated the output of penicillin in the urine with the concentrations in the blood during intravenous infusions in man given at different rates. In spite of wide variations in various reports, all the oral penicillins, as seen in Table III, page 63,

Table II. Peak Serum Levels Averaged From Published Sources* Expressed as Units or Micrograms per ml. (200,000^u = 125 mg.).

Oral Product			Fasting	Post-Prandial
Penicillin G potassium	Tablet	200,000^u	0.4^u	0.2^u
	Capsule	200,000^u	0.6^u	-
	Tablet	400,000^u	1.2^u	-
	Powder in Water	400,000^u	1.6^u	0.4^u
G benzathine	Tablet	200,000^u	0.1^u	0.06^u
	Aqueous Suspension	300,000^u	0.2^u	-
Penicillin V acid	Tablet	200,000^u	0.5^u	0.3^u
	Tablet	250 mgm.	2 ug.	1.5 ug.
V potassium	Tablet	250 mgm.	2.5 ug.	2 ug.
V benzathine	Aqueous Suspension	300,000^u	1^u	-
	Aqueous Suspension	250 mgm.	-	1.5 ug.
Phenethicillin	Tablet	250 mgm.	3.5 ug.	2 ug.
	Tablet	500 mgm.	6 ug.	4.5 ug.
Ampicillin	Capsule	250 mgm.	2.5 ug.	-
	Capsule	1 gram	7 ug.	-
Cloxacillin	Capsule	500 mgm.	7 ug.	5 ug.

* (Wright et al. 1953, Jones and Finland 1955, Putman et al. 1955, Peck and Griffith 1955, Juncher and Raaschou 1957, Morigi et al. 1959, Cronk et al. 1959, Knudsen and Rollinson 1959, Keen and Mathews, 1960, McCarthy and Finland 1960, Williamson et al. 1961, Knudsen et al. 1962, Bond et al. 1963, Stratford 1963).

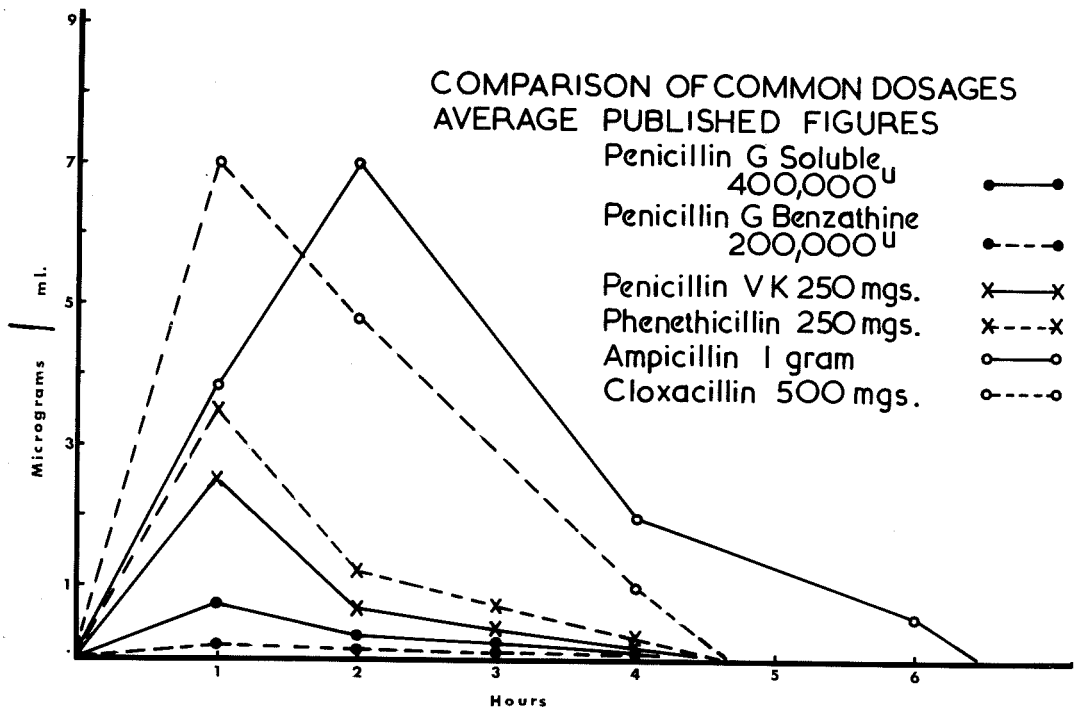


Figure 1. Oral penicillin blood level curves.

generally show this linear relationship of urine excretion to serum levels except for phenoxybenzyl penicillin, which shows a lower excretion relative to high serum levels. The recovery of penicillins from urine is always incomplete, the greater part of intravenous doses appearing within 1-2 hours and lesser amounts of the oral doses within 3-6 hours. The latter apparently expresses the amount of oral absorption, the remainder passing out with and being partly destroyed in the faeces. Any tissue destruction or formation of biologically inactive metabolites or extra-renal excretion in bile (except for ampicillin and cloxacillin) is probably not significant at low oral levels. As greater urine recoveries are found with the more highly protein-bound penicillins, one must postulate that this is not only a reflection of their better absorption and higher total blood levels, but that there is no real delay in the dissociation of protein-bound penicillin in the blood of the renal circulation. Phenoxybenzyl penicillin is an exception, as its very high rate of binding is sufficient to explain its low urinary excretion (Heatley 1956, Pindell et al. 1959, Kunin 1961, and Rollo et al. 1962).

Table III. Urine Excretion Range of Oral Penicillins Over 6 Hours, Expressed as Percentages of Dose Administered.
Average Peak Serum Levels in Micrograms per ml. from 250 mg. doses from Published Sources.*
Average Protein Binding as Percentages.

	Protein Binding	Serum Levels	Urine Excretion
Penicillin G potassium	50	1	12 - 20
Penicillin V acid	80	2	30 - 45
Phenethicillin	80	3.5	35 - 55
Phenoxybenzyl	97	6	20 - 25
Ampicillin	10	2.5	30 - 40
Cloxacillin	High	3.5	30 - 40

* (Tompsett et al. 1947, Brown et al. 1956, Cox et al. 1956, Smith et al. 1956, Cronk et al. 1959, Knudsen and Rolinson 1959, McCarthy and Finland 1960, Rolinson and Stevens 1961, Stratford 1962, Knudsen et al. 1962, Rollo et al. 1962, Bond et al. 1963).

Diodrast, known to be excreted by the renal tubules, was shown to have a similar rate of elimination to penicillin, and this suggested not only glomerular filtration (the normal filtrate being 20% of the renal flow), but additional excretion via the renal tubules. Diodrast did, in fact, lessen the excretion of penicillin when both were administered together by Rammelkamp and Bradley (1943). Experiments with amino-hippuric acid were followed in 1947 by the use of the uricosuric agents caronamide and then probenecid, the successful competitive inhibition of renal tubular excretion resulting in 2-4 fold increases in serum levels for oral penicillins, with about a 2 hour prolongation of the therapeutic range (Crosson et al. 1947, and Boger et al. 1950). Later tests also showed that probenecid effectively blocked the newer penicillins, but serum accumulation has not been noticed with normal dosage schedules (Diding and Frisk 1959, Trafford et al. 1962, and Klein et al. 1963b).

Recent chromatographic examinations of penicillin in urine (Bond 1964), have revealed up to 20% of at least 2 metabolites for each phenoxypenicillin administered. The biological activities of these substances differed only slightly from the parent substance. It is not known to what extent the kidney tubules are responsible for this, but small amounts of metabolites of phenethicillin and cloxacillin have been found in the blood, presumably originating from the liver. The only importance attached to these find-

ings appears to have resulted in theoretical arguments over the validity of comparative urine assays.

Penicillin excretion in bile has been proved both in animals and man (Abraham et al. 1941, and Struble and Bellows 1944). Excretion by the liver in large amounts has been shown for ampicillin, where 22 micrograms per ml. have been assayed from aspirated human bile compared with 7.5 micrograms in the serum (Stewart and Harrison 1961). High levels of cloxacillin have also been found in the bile of rats (Nayler et al. 1962). Penicillin has been detected in the saliva of animals following intravenous doses, but not following oral administration (Adler-Hradecky and Kelentey 1963). It has also been detected in human sputum after parenteral therapy (Humphrey and Joules 1946). Small amounts of penicillin have been assayed in the milk of nursing mothers (Greene et al. 1946), and sweat has been noted not to contain oxacillin (Hewitt 1963).

Penicillin Inactivation.

The rates of destruction of commercial penicillin have been quite variable, but Benedict et al. (1946) found a half life of about 60 hours using various buffers including phosphates at pH 7.5 and 37°C. Bigger (1944a), found a 5% per hour loss in serum at 37°C., and Bond et al. (1963), has reported a 10-20% loss in 5 hours with the phenoxypenicillins in serum. The results of my experiments are shown in Table

IV, page 67, the inactivation figures having been averaged from 3-4 tests incubated in 1-4 ml. volumes in test tubes. This was followed by a plate diffusion assay for each type of test fluid and for each of the several different test sera.

Percentage losses were quite considerable after overnight incubation, particularly in small volume tests, and there was a definite variation in individual results, this being far greater in serum than in broth. Individual and pooled unheated sera were used because previous tests with sera heated to 56°C. for half an hour did not reveal any obvious differences. Increased losses were noted with unadjusted sera at pH 8 or more, and adjusted sera were found to be unstable at physiological pH with readings of greater than pH 8 after overnight incubation at 37°C. Ampicillin also decayed more rapidly in phosphate buffer than in serum or nutrient broth. Changes in serum pH and differences in serum amidase content, as well as technical mistakes in not mixing adequately and not standardising test volumes in relation to surface areas exposed to oxygen, probably explain most of these variables. Considerable losses were also recorded in 25-50% serum broth tests.

The implications of these findings are considerable, as will be seen in Chapter VI, where these amounts of penicillin destruction are shown to be very important, quite apart from serum binding effects. Inactivation by bacterial pen-

icillinase is discussed under penicillin resistance on page 132.

Table IV. Average Inactivation as Percentages over 20 hours. Nutrient Broth, Phosphate Buffer and Serum at pH 7.4 and 37°C.

	Broth	Buffer	Serum
Penicillin G potassium	25	30	60
Penicillin V acid	30	40	80
Phenethicillin	30	45	85
Ampicillin	40	80	40
Cloxacillin	30	50	85

Serum Protein Binding.

An effect of serum or blood on penicillin apart from direct destruction was first noticed by Rammelkamp and Keefer (1943b), when they found some of their tube-dilution anti-staphylococcal and antistreptococcal assays enhanced by added serum or blood. Holmes and Lockwood (1944) then reported that human, horse and rabbit sera interfered with the anti-bacterial action of penicillin in their 2 hour staphylococcal turbidimetric tube assays, and they postulated a rapid-acting anti-penicillin factor, but this has never been substantiated. Serum impairment of staphylococcal inhibition zones in plate assays of penicillin was reported by Richardson et al. (1946), and serum binding with consequent inactivation of the bound

portion was thought to be the explanation. Rapid penicillin destruction was thought to be excluded by an apparent 100% recovery in tube dilution assays performed in broth on the same material. Because sulphanilamide was known to be bound by serum protein, Chow and McKee (1945) mixed penicillin with separate human serum albumin and gamma globulin fractions and tested them for binding in a static dialysis experiment. The cellophane bag with albumin complex always assayed more penicillin than the phosphate buffer dialysate, and they even precipitated and recovered the apparently bound active complex as a dried powder. Tompsett et al. (1947) then found that the extracted natural penicillin K was highly serum bound, and although in vitro tests had previously showed it to be very active against Gram positive organisms in broth, in vivo tests in infected mice by Eagle and Musselman (1946) gave very poor responses. This again appeared to give proof of lack of activity in the bound portion, destruction of penicillin K in the body not being accepted as sufficient to explain the discrepancy.

Dialysing experiments have now established beyond all reasonable doubt that serum albumin binds the different penicillins to varying degrees (Ullberg 1954; Smith et al. 1956; Kunin 1961; Scholtan and Schmid 1962; Bond et al. 1963). The techniques have utilised static dialysis with microbiological or radioactive total assay of serum-penicillin samples and their dialysates, or ultrafiltration with direct assays

of filtrates containing free unbound penicillin.

Gel adsorption and filtration through sephadex, and subsequent assay of free penicillin in the elutes, has shown similar binding effects (Scholtan 1964). Acred et al. (1963) filtered serum-penicillin through sephadex columns equilibrated with water, assaying the filtrates and measuring the protein optical densities. Using relatively large amounts of penicillin they showed some apparent loose binding with penicillin G. They also confirmed that serum albumin was the factor binding the penicillin, by protein fractionation of the complex with saline of graded molarities, and subsequent assay and electrophoresis. Dolkart et al. (1948) had previously questioned the binding concept because of negative fractionation results, but dissociation would appear to have taken place during their precipitation processes.

Recent proof of serum-protein binding has come from tests revealing a certain amount of inhibition or displacement of bound penicillin by analogues of the R side-chain of the penicillins (Kunin 1965).

The now well known inhibitory effect of serum in penicillin tube dilution and plate diffusion tests has generally been accepted as indicative of penicillin binding and the biological inactivity of this portion (Gourevitch et al. 1959; Smith et al. 1956; Colville and Quinn 1961; Kunin 1964). This inhibition shows an apparent correlation with the binding

of different penicillins (Quinn 1964), and a decrease in penicillin activity has been shown with increasing protein concentration (Eagle and Tucker 1948; Smith et al. 1956). These observations seemed to lead to the belief that broth dilution of serum-penicillin resulted in dilution dissociation of the bound portion, whereas serum dilution did not (Tompsett et al. 1947; Robinson 1964).

Original Binding Studies.

The binding reaction:

Dr. J. R. Coulter and I have made a combined study of penicillin binding, and we contend that chemical mass-action constants necessitate differing amounts of dissociation in both diluted broth and serum penicillin mixtures. Klotz et al. (1950) demonstrated the reversible nature of protein binding, by finding that equilibrium rapidly re-established itself in a static dialysis experiment, every time the buffer dialysate was successively drawn off and replenished. It seemed to us that the binding reaction would obey the law of mass action like any other reversible reaction.

Free Penicillin + Protein = Complex (bound penicillin)

$$\frac{[\text{Pen}] \times [\text{Protein}]}{[\text{Complex}]} = K$$

where [Pen] = concentration of free penicillin etc.

K = a probable series of constants for multiple protein binding reactions.

If the bound complex of penicillin in serum is now diluted in tubes of broth, the free penicillin and protein would seem to be diluted equally, with the complex decreasing at a different rate to keep the constant K. This would mean that there was a certain amount of dilution dissociation in broth. In actual experiments Verwey and Williams (1962) claimed that variations in small amounts of penicillin G and V, in the relatively large protein reservoir of canine serum, did not reveal measurable differences in the degree of binding. They did show, however, an exponential increase in binding as the percentage of serum protein was raised. Bond et al. (1963) then reported an apparent smaller binding degree at lower serum levels for the common phenoxy-penicillins in a large number of human volunteers.

In the mass action equation, the protein concentration in a serum diluent will be in relative excess over therapeutic levels of penicillin, so that the protein will virtually remain another constant.

$$\frac{[\text{Pen}]}{[\text{Complex}]} = K_1$$

This means that dilution of complex in serum would again keep a constant equilibrium, and that decreasing penicillin concentrations would again result in a decrease in the degree of binding. Even though binding percentages have been reported as a specific figure for individual penicillins, extrapolations from infinity in linear graphs should show a slight

slope instead of an upright line. Scholtan and Schmid (1962) did in fact plot this slope. We however have also demonstrated that penicillin G, diluted equally and in parallel with serum and broth, still shows a definitely smaller S. lutea inhibition zone with serum than it does with broth. The explanation is apparently the difference in dilution dissociation, and the interference to the diffusion of penicillin released from the reservoir of bound drug left behind in the agar cup. This is only loosely bound, but diffusing free penicillin is probably rebound and released in a dynamic movement through that small portion of serum protein that has itself diffused into the agar.

Inhibition of 18 hour tube dilution tests by serum, as against plate diffusion assays, is not to our way of thinking so much a reflection of serum binding and less dissociation, but a result of penicillin inactivation from serum enzymes and inhibitors. In some cases there is also a direct inhibitory effect of serum on the growth of the test organism itself, and the bactericidal efficiency of penicillin (Quinn 1964; Chapter VII of this thesis). Tompsett et al. (1947) dismissed the effect of penicillin inactivation because of widely varying test results, but Wick (1964) has confirmed our own work in this regard with tests on cephalothin and other antibiotics. A failure to show the inhibitory effects of added serum albumin in tube sensitivity tests was published by Bringhurst and Marcus (1961), but a repetition of the work

disclosed altered staphylococcal growth characteristics and invalid titre readings.

Lymph and Tissue Binding:

On considering *in vivo* effects, serum protein binding would seem to partly govern early tissue diffusion, and this must depend on the looseness or firmness of the bond. Free penicillin would immediately diffuse, and this would be followed by a variable dissociation of bound drug to keep a constant equilibrium. Actual tissue assays of homogenised laboratory animal organs have shown good levels of the commonly used penicillins, and definite variations have been observed in the levels in different tissues. Some of these appeared to be correlated with serum binding, similar to sulphonamides, but liver and kidney extracts always assayed high penicillin levels even after only one hour from dosing (Richardson *et al*. 1946; Acred *et al*. 1962; Barber and Garrod 1963; Kunin 1965). Serum and tissue protein binding have been compared with serum and tissue assays for individual penicillins in mice, and the tissues have shown higher levels of free drug with lower binding (Scholtan and Schmid 1962). Direct lymph assays and binding tests in dogs have also revealed some inverse relationship between plasma binding and lymph to plasma assay ratios, but calculated free lymph levels were higher than blood (Verwey and Williams 1962). Direct total lymph assays were then found to be higher than those of blood in rats, which were injected with different

penicillins (Brown 1964).

Rolinson (1964) has postulated that only the free penicillin in the blood governs the free penicillin in the lymph and tissues, in a system under dynamic equilibrium, and that free lymph and tissue levels could never be higher than the peak free levels in the blood. We point out, however, that the blood and lymph of dogs, rats and humans contain roughly equivalent amounts of protein, and that total lymph levels of penicillin were shown to be close to or higher than blood in dogs and rats. This means that even human lymph, with less than half the protein of blood, must have much higher free penicillin levels, and that a large portion of the total penicillin in the blood must quickly dissociate and become available for diffusion, over the time that a blood level is held. The different binding potential seen in different species would not seem to alter this basic concept.

Criticism must be levelled at the previous lack of adequate controls in dialysis studies. Unstable pH both of serum and dialysate buffers has, in our opinion, invalidated the commonly reported binding figures, and some of the ultrafiltration work has not controlled the dissociation of loosely bound penicillin in systems drawing free drug into filtrates of a relatively large volume. We have conducted dialysis and gel filtration studies of our own, and more careful control has revealed different binding percentages

for penicillins in serum, at varying temperatures and pHs. We have also confirmed the very loose nature of most penicillin binding, even at therapeutic blood levels.

Dialysis Experiments:

We experimented with a static dialysis method using standard benzyl penicillin (penicillin G), phenoxymethyl (penicillin V), phenethicillin, ampicillin and cloxacillin. Serum was separated from the freshly drawn blood of various people, and it was noted that the pH rapidly rose to above pH 8. Ordinary buffers were also found to be unstable under dialysis, and a special one of known ionic strength and stable pH was prepared. KCl 5mm mol, Na_3PO_4 0.6 m mol, NaCl 93 m mol and $NaHCO_3$ 45 m mol were equilibrated at 4^oC. with a mixture of 5% CO_2 and 95% O_2 to give a pH of 7.4. Variations in the NaCl, $NaHCO_3$, CO_2 and O_2 gave any desired stable pH at any temperature.

Separate buffer and serum aliquots of 10 ml. were prepared with final concentrations of 20 micrograms/ml. of penicillin. Fresh and incubated mixtures were placed in dialysis bags, equilibrated with the gas, and then suspended in 20 mls. of continuously stirred and gas-bubbled buffer. Half hourly dialysates were then sampled over 4 hours with assays at various temperatures and pHs. These were again subjected to pH measurement and S. lutea plate assay, using phosphate buffer to dilute the standard penicillin (see Chapter VI). The dialysed serum was ignored because of inconsistencies in

assaying correct total recoveries. The rise of penicillin concentration in the buffer and serum dialysates was then plotted against time, and serum binding was calculated from the differences at equilibrium (Figure 2, page 77).

Penicillin decay in buffer was excluded by testing a control 30 ml. of gas-bubbled buffer and penicillin. Possible serum inactivation of penicillin was excluded by previous tests showing no significant destruction of penicillin over at least the first 3-4 hours with the sera used. Equilibrium had in fact been achieved after 1-2 hours with buffer and 2-3 hours with serum, and dialysate assays at continuing equilibrium showed no evidence of short-term penicillin decay. Protein leakage from the dialysis bags was also excluded by spectrophotometric readings at 280 mu. Protein interference with dialysis was taken care of by noting the difference in the time of establishment of the above equilibrium states.

For gel filtration we separated serum-penicillin complex in sephadex G 25 columns, with the hope of showing two peaks of antibiotic activity as an indication of penicillin binding. The first peak would be expected with the protein, and the free penicillin would follow after, as was the case for the large dose penicillin separations of Acred et al. (1963). If the rate of dissociation of the complex was fairly rapid compared with its descent in the column, only one isolated free peak, with a tail skewed toward the earlier

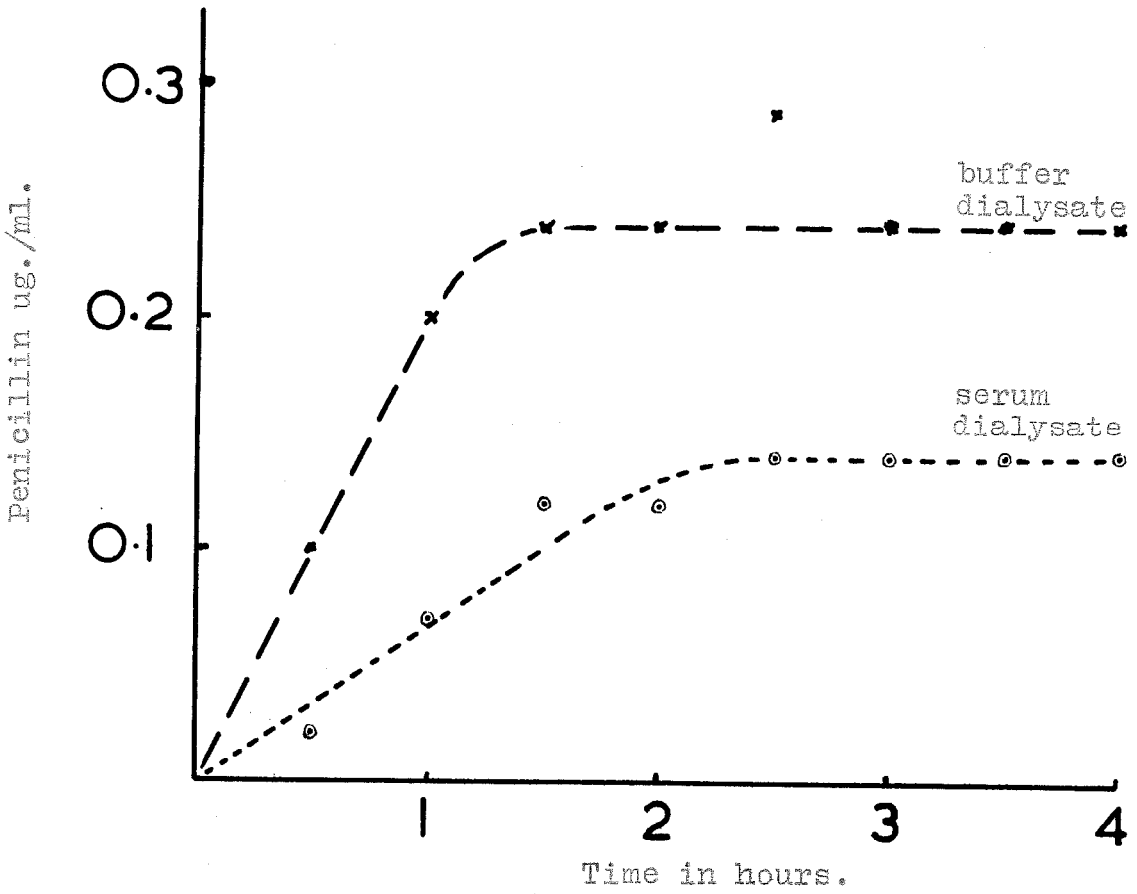


Figure 2. Phenoxymethyl penicillin dialysate curves at pH 8.5 and 37°C.

Buffer dialysate x----x

Serum dialysate o----o

protein, would be expected.

All the penicillins were applied to the 36 cm. column in 0.4 ml. volumes at 20 micrograms/ml. concentrations, except for cloxacillin when 200 was used. Experiments were conducted at 4°C., 20°C., and 37°C., with a flow rate of bicarbonate-CO_2 buffer varying from 0.5 - 0.75 ml./minute, and fractions were collected in 0.5 ml. volumes. These were assayed on S. lutea plates and measured spectrophotometrically at 280 mu. for protein content.

Absorption to wet sephadex was not significant, as approximately 100% of penicillin was assayed from the filtrates. The assumption that penicillin was bound at the beginning of the experiments was postulated from the fact that sephadex filtration of previously dialysed bound complex produced identical results. Dissociation on the column may take place, but this would be a measure of the looseness of the binding. The skewed curves in the case of firmly bound penicillin not only confirms the fact of binding but also proves that passage through the column does not split off all protein bound drug.

The results of dialysing penicillin G and phenethicillin over the pH range 6.9 - 8.9 at 4°C, showed a considerable increase in the degree of binding of both penicillins as pH was raised above physiological limits. This result did not arise from alterations in ionic strength, as our previous tests of this showed no difference in binding. The

individual dialysis experiments are listed in Table V hereunder, and it can be seen that penicillin G and ampicillin showed hardly any measurable binding at 37°C. Penicillin V revealed a definitely increasing degree of binding with rising pH at 37°C, but the higher binding of phenethicillin was constant.

Table V. New Protein Binding Percentages. Individual Experiments.

Penicillin Type	Temperature	pH	% Penicillin Bound
Penicillin G	4°C	6.93	30%
"	"	7.30	20%
"	"	7.40	25%
"	"	7.48	32%
"	"	7.55	58%
"	"	8.93	58%
Phenethicillin	4°C	6.93	31%
"	"	7.38	34%
"	"	7.40	59%
"	"	7.43	60%
"	"	7.45	73%
"	"	8.95	75%
Penicillin G	37°C	7.56	< 10%
"	"	7.63	< 10%
Phenethicillin	37°C	7.22	75%
"	"	7.22	73%
"	"	7.27	75%
"	"	7.43	60%
"	"	7.59	73%
Ampicillin	37°C	7.40	< 10%
"	"	7.43	< 10%
Phenoxymethyl Penicillin	37°C	7.38	36%
" "	"	7.63	58%
" "	"	8.51	68%
Cloxacillin	37°C	7.28	86-95%
"	"	7.46	93%

Even though there is some individual variation with different sera, these results indicate that protein binding is less than that previously reported, within the physiological pH range of 7.2 - 7.4. The pH of ordinary dialysis tests has in our experience quickly risen above physiological levels, and binding data has consequently been inaccurate, except for highly bound drugs such as cloxacillin. Ultrafiltration would seem to suffer from the same effect since separated serum quickly rises in pH and this would be further aggravated by negative pressure drawing off more CO_2. Scholtan and Schmid (1962) reported that benzyl and phenoxymethyl penicillin were less bound at higher temperatures, and our results confirm this except for phenethicillin.

Gel Filtration:

Sephadex filtration of serum-penicillin complex revealed two peaks of U.V. absorption. The first contained 90% of the protein coming through 8 ml. after application, and the second 21 ml. later. Penicillin appeared between the two, peaking 12 ml. after the main protein peak. All our test penicillins appeared as free drug unassociated with the proteins, but cloxacillin gave a skewed peak tailing off toward the protein. Figure 3, page 81, depicts one of our experiments where A represented penicillin G, B phenethicillin and C cloxacillin. Penicillin in buffer was put on the 37°C. column at the test serum pH of 7.9, and this was immediately followed by the fresh serum. In this way the protein

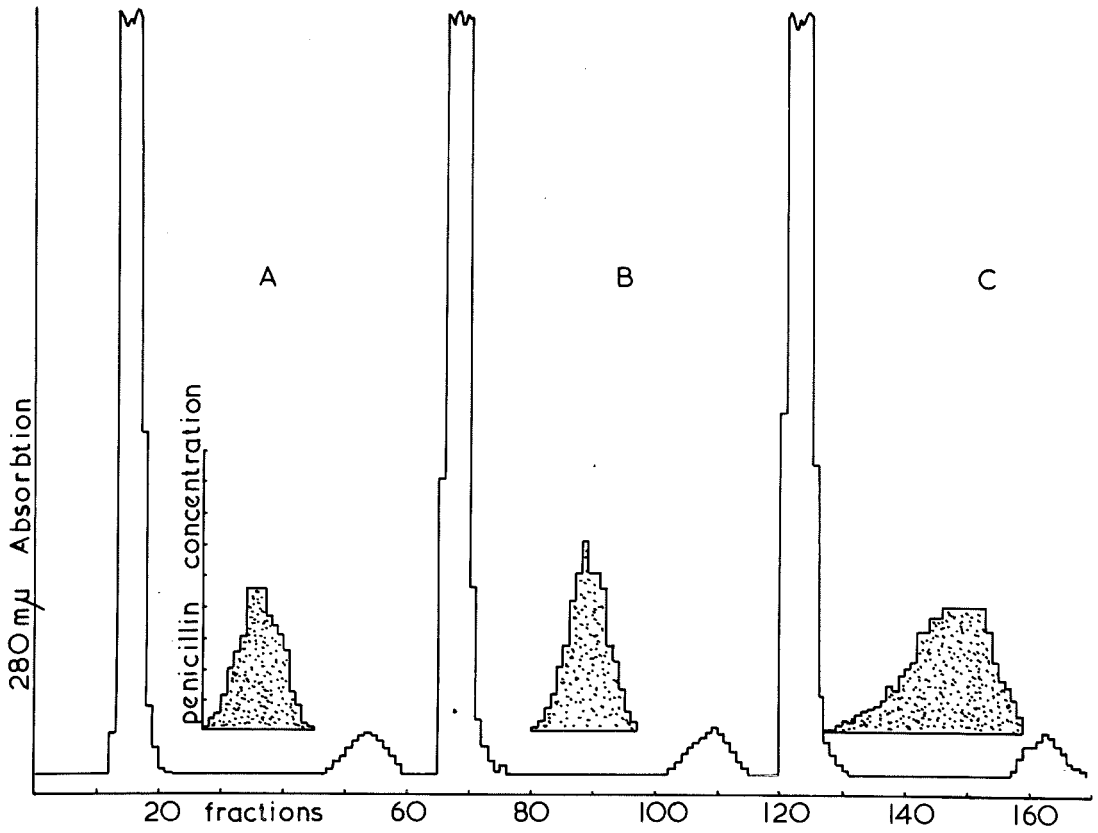


Figure 3. Experimentally derived spectrophotometric curves for serum protein - penicillin separation on Sephadex G25.

A penicillin G

B phenethicillin

C cloxacillin

was made to run through the penicillin in the column, which was flowing at 0.6 ml./minute for the collection of 0.5 ml. fractions. Following this, penicillin incubated at 37°C. for 15 minutes was run through, and substantially the same curves recovered.

It would appear from this that protein binding was very loose, and dissociation occurred very rapidly in the case of penicillin G and phenethicillin. Highly bound cloxacillin, on the other hand, was skewed toward the protein, and this was probably due to firmer binding and a slower dissociation of the bound complex.

From this it seems evident that common penicillins are variously bound to serum protein in a loose complex, and the resultant effects *in vivo* are probably of little significance in regard to free diffusion and tissue levels of antibiotic. Further discussion of *in vivo* significance is found in Chapter VII, page 119, and of *in vitro* in Chapter VI, page 102. We also hope to publish a paper concerning these original binding studies and the discussion of their significance.

Tissue Inactivation.

Finally, some direct tissue destruction of penicillin G has been shown in homogenised extracts of the livers, lungs and muscles from experimental animals (Richardson *et al*. 1946 and Randall *et al*. 1947); and Spitzy (1955) reported that penicillin V was inactivated more rapidly than G, indicating

that some tissue destruction would be expected in man. No obvious loss of penicillin activity has been found in pus (Garrod 1945), but Few et al. (1952) showed that some bacteria bound a certain amount of penicillin to the cell wall. Penicillinase production would also be expected from resistant strains of pathogenic staphylococci co-existing with the normal flora of the skin, naso-pharynx and urethra, and there is evidence that penicillin is in fact destroyed to some extent in these sites (Sanders et al. 1962).

Tissue Distribution.

This subject has been investigated directly and indirectly, and it has been difficult to arrive at valid conclusions. Direct assays of commercial penicillin in homogenised tissues from experimental animals, and correlations of plasma and tissue levels, have shown the kidney to contain amounts in excess of the plasma, with liver and lungs at about half and muscle and spleen at about a quarter of the plasma levels. No account has been taken of the contribution of tissue fluids or the binding effect of tissue proteins (Richardson et al. 1946, and Bond 1964). Direct but artificial tests with fibrin clots inserted subcutaneously in rabbits have shown that penicillin was assayable in the clots for several hours longer than in the blood (Weinstein et al. 1951). S^{35} labelled radioactive benzyl penicillin has given an autoradiographic picture of rapid general body

distribution in mice, including *in vitro* proof of good intracellular diffusion into leucocytes and, to a lesser extent, into erythrocytes, (Ullberg 1954). More recent studies by Acred *et al*. (1962) have also revealed good assays of ampicillin from all organ extracts in rats.

Diffusion into human body fluids varies, the penicillins in general showing only minute amounts in normal cerebrospinal fluid, with meningeal infections definitely raising the level (Janowitz *et al*. 1948, Stewart *et al*. 1961, and Ivler *et al*. 1963). Penicillin penetrates with difficulty into the eye, and often requires subconjunctival injection, (Sorsby and Ungar, 1946). Good diffusion into lymph, wound exudates and abscesses has been shown by various workers, including adequate and rapid crossing of the placental barrier, and some diffusion into pathological fluids such as pleural, pericardial, peritoneal and joint exudates (Florey *et al*. 1949).

Indirect investigations of the comparative distribution of the different penicillins have depended upon an assessment of serum binding effects, excretion rates and calculated relative distribution volumes and serum half lives. Some of the new penicillinase-resistant isoxazolyl penicillins also show unusual properties such as good fat solubility and so the concept arises of temporary body-fat depots (Wahlqvist (1964). Urinary excretion rates are, in general, the same and low levels can be measured for 12-24 hours follow-

ing a dose, even though blood levels have disappeared after 6-8 hours. This is partly explained by the time taken to pass through the urinary tract.

Radio-active penicillin studies have been inconclusive concerning tissue retention, but it would appear that low, and probably ineffective levels do persist in certain tissues for prolonged periods, even though Eagle et al. (1953) proved the presence of effective concentrations of penicillin in infected muscle foci of mice only at the time of effective serum levels. Serum protein binding and its rapid rates of association and dissociation with most of the common penicillins would appear to only briefly govern the free penicillin available at therapeutic levels for tissue diffusion, and/or for take-up by bacteria. Lymph and extracellular fluid proteins would likewise be available as an inexhaustable buffer and a readily available reservoir while blood levels were maintained.

Relatively high blood levels have been claimed to be an indication of poor tissue diffusion on the basis of comparative and equal intravenous injections. Spitzy and Hitzenberger (1957) used a concept of distribution volume whereby the decrease in serum levels was considered in two phases; the first probably being the development of a steady state of equilibrium between the plasma, the tissues and the excretory mechanism; the second and slower clearance phase being the sole effect of excretion. Penicillin regression

lines on semi-logarithmic graphs reveal serum levels of ~~oral~~ penicillin V to be lower than those obtained with equivalent intravenous doses of penicillin G, the inference being that more V is taken into the tissues. Equations based on these regression lines also give an estimation of relative distribution volumes and serum half lives, with V having a higher distribution volume but a lower half life than G. Kunin (1961) has since examined four penicillin analogues and finds penicillin V to have a shorter half life than either penicillin G or phenethicillin, but with no significant differences in relative distribution volume. He concludes that these relative differences are not important.

Price et al. (1960) performed experiments suggesting that different body masses and flow rates, through different animal organs and tissues, might influence the redistribution of drugs in the body. However, good therapeutic responses with the penicillins in individuals of varying body weight, together with phenethicillin studies on children by Kneebone and Maxwell (1960), seem to deny this. Finally, Kharchenko (1963) has stressed the fact that body protein is needed for the transport of penicillin and other small molecule substances, but free diffusion gradients and permeability would also seem to be factors in explaining tissue distribution at the capillary level, particularly for penicillins with little or no C.S.F. penetration, despite small amounts of protein being secreted into the fluid.

Therapeutic Effectiveness.

In vivo studies have been conducted in experimental animals as a guide to therapeutic potential in man, and the comparative CD_{50} values have been estimated (the dose curing half the test animals). Absorption, serum binding and other parameters, however, are not the same as in man, and it appears that such studies merely select the best drug for the particular test animal, and do not permit similar conclusions in man. An example of a successful study is that of Gourevitch *et al.* (1959) who found phenethicillin quite efficient in mouse protection tests against staphylococci.

Toxicity.

This has been proved to be low, with only mild gastrointestinal and skin disorders for all oral penicillins. Very rare neutropenias and oligo-haematurias have been observed with methicillin, and some bone-marrow depression and hepatocellular dysfunction with oxacillin (Stewart 1964). Some heightened muscular irritability has been noticed in uraemics and occasional meningeal irritation in a few cases injected intrathecally with benzyl penicillin (Barber and Garrod 1963). Penicillin therapy has resulted in some superinfections with resistant staphylococci, pathogenic yeasts and Gram negative bacilli, the latter particularly with the penicillinase-resistant isoxazolyl penicillins (Hewitt 1963, Bunn and Milicich 1963). The problem of hypersensitivity is a real one

and is discussed in a later chapter on clinical assessment.

Animal tests have shown the penicillins to be remarkably free of toxicity, LD_{50} estimations (the lethal dose for 50% of the test animals) revealing the steady improvement of the products as purity has increased (Rake and Richardson 1946). Penicillin G and V both exhibit minor toxicity for mice, and dogs have tolerated large doses of penicillin V for 2 months with no visceral or haemopoietic damage (Anderson et al. 1955). Acute and chronic toxicity tests with phenethicillin and skin irritation studies on animals have shown its safety, and large intravenous doses in dogs have had no effect on blood pressure, autonomic reactivity or respiration (Pindell et al. 1959). All the later penicillins are likewise free of toxicity, and in human trials upwards of 20 megaunits daily of benzyl penicillin has produced no toxic effects in early tests (Hunter 1947).

Mode of Action.

The penicillins are bactericidal antibiotics, viable counts showing marked drops in bacterial test populations in 2-3 hours, usually with lysis (Hobby et al. 1942). Bacteria were shown to be killed more rapidly by penicillin in the growth phase (Hobby and Dawson 1944), and small numbers of staphylococcal "persisters" were discovered by Bigger (1944b) in penicillin sterilising tests. Absorption of S^{35} labelled penicillin by penicillin sensitive bacteria was demonstrated

by Cooper and Rowley (1949), through the disappearance of small amounts of radioactivity from culture filtrates. Few *et al*. (1952) then obtained proof of the take-up of radioactive penicillin by the bacterial cell wall.

Recent evidence suggests that penicillin interferes with the incorporation of aminoacids into the cell wall of the growing bacterium, and thus with the synthesis of cell-wall mucopeptide. Among others, Lederberg and St. Clair (1958) showed that *E. coli* and *S. typhimurium* formed labile bodies, later termed spheroplasts, when transferred to a penicillin broth. When the penicillin was removed, about half the bodies reverted to normal rods. Mucopeptides were then shown to be present in bacterial cell walls and their formation proved to be influenced by penicillin (Park 1958, Mandelstam and Rogers 1959).

Cellular body defence mechanisms appear not to be involved in the first rapid bactericidal effects of penicillin *in vivo* (Gowans 1953), but the prolonged therapy sometimes needed for the sterilisation of infected bone and deep-seated abscesses indicates that both macrophages and immune bodies are the ultimate deciding factors.

CHAPTER V.

PHARMACEUTICAL INVESTIGATIONS.

Oral Dosage Forms.

These consist of compressed plain or film coated tablets, plain or coloured gelatin capsules, aqueous or coconut and peanut oil suspensions, and syrups. Powders or granules combined with flavours, dyes and sweeteners for reconstitution in various fluids are also employed. The pharmaceutical preparation of these oral penicillins depends on the chemical composition and properties of each product, and this in turn is determined by the different side-chains attached to the penicillin nucleus.

The stability of the penicillins in the solid state depends to some extent on the moisture content, and limits of moisture are set down for the individual products. Tablets of penicillin G packaged with silica gel were found by Stephenson and Foster (1948) to be more stable than those without the drying agent. Insoluble salts of penicillin G and V, such as benzathine and hydrabamine, are stable as dry powders for upwards of 3 years at room temperature (Buckwalter 1954), and pure soluble salts are much more stable in compressed tablet form, and quite stable if also hermetically sealed (Buckwalter and Holleran 1953).

In liquid form, hydrolysis of soluble salts to penicilloic acid is fairly rapid and, as the reaction is cat-

alysed by both acid and base, greater stability is found with added buffering agents (Clapham 1950). Two weeks at refrigerator temperature is, in fact, the maximum permissible time for keeping solutions of penicillin G, and some of the newer penicillins only keep their potency in solution for about one week or less. The sparingly soluble amine salts of penicillin G and V have, however, permitted the production of aqueous or oily suspensions fully stable for a year and eighteen months respectively at optimum pH levels, and with low solubility suppressing the unpleasant taste they have found a definite paediatric use, together with powders, granules and syrups (Keen and Mathews 1962, Schwartz and Buckwalter 1962).

Some clinicians blame oily suspensions for troublesome diarrhoea in children, but better suspensions are continually being developed, and the latest penicillins are also being prepared in various paediatric forms. Thought is given to such factors as stability, taste, particle size (the fine particle apparently giving more rapid absorption), and water content (penicillin V with a low inherent water content showing discolouration of aqueous suspensions if the water content is too high).

The convenience and stability of compressed tablets has, however, made this dosage form the most useful, except where capsules have been given as an alternative for psychological, taste or dyspeptic reasons. Film tablets coated

with corn gluten derivatives came into use in 1958, with claims for smaller tablets, less damage from packaging, faster dissolution times and greater ease in swallowing (Martin and Cook 1961). Experience has shown that the ease of swallowing is the only real advantage and that absorption is, in fact, often more irregular.

Tablet Disintegration Tests.

The firmness of compression and the amount of binding material present in a tablet are generally considered to be important with regard to reliable absorption. Wright et al. (1953) and others found that lightly compressed tablets disintegrated more rapidly than hard tablets, and that they gave better absorption. Buffered penicillin G tablets with a disintegration time of 30-45 minutes gave somewhat higher peak levels and more constant absorption than unbuffered tablets, and hard tablets of benzathine penicillin G with a disintegration time of 1½ - 2 hours were absorbed more erratically than softer tablets disintegrating in 10-45 minutes. Jones and Finland (1955) found a slowly disintegrating tablet of penicillin V slightly superior to a soft product when given before breakfast, but inferior when taken after the meal.

The oral tablets found on the Australian market were tested by me. In this study a modified British Pharmacopoeia method was used, placing individual tablets in a test tube of water or other test fluid, leaving about half an inch of air

space. The tubes were stoppered and repeatedly inverted on a rocker table at such a speed that the tablet did not strike the ends of the tube. The upper limit of time taken for the disintegration of an acceptable tablet was 20 minutes. Table VI shows the results of my tests conducted on non-expired plain and film coated tablets rocked in warm water, artificial 0.1% HCl gastric juice and an acid-pepsin mixture.

Table VI. Average Disintegration Times in Minutes. Fractions Not Disintegrated in 20 Minutes in Brackets.

	Water	Acid	Acid-pepsin
Penicillin G			
benzathine (200,000^{u} Wyeth)	(1/3)	(1/3)	(1/3)
benzathine (200,000^{u} C.S.L.[1])	2	2	-
Penicillin V			
calcium (250 mgm. C.S.L.)	2	-	-
potassium (125 mgm. Sigma)	20	-	-
potassium (125 mgm. film Abbott)	10	(1/2)	(1/2)
potassium (250 mgm. film Abbott)	10	(1/2)	-
Phenethicillin			
potassium (125 mgm. C.S.L.)	15	15	15
potassium (250 mgm. C.S.L.)	15	15	15
potassium (250 mgm. Sigma)	15	15	15

[1] Commonwealth Serum Laboratories.

Most of the tablets tested were obviously satisfactory and even the harder partly undissolved tablets and the acid resistant film-coated tablets gave comparable absorption figures.

Potency Tests.

I have conducted these, together with long-range stability studies on various oral penicillins currently available in Australia, all products being within the expiry time or close to time of manufacture. A microbiological assay has been used to avoid chemical measurement of antibiotic and any biologically inactive degradation products. The standard plate assay method of Grove and Randall (1955) was chosen, utilising a Staphylococcus aureus (NCTC 6571) in a two-dose procedure. Standard penicillin doses of 1 and 0.25 units or micrograms per ml. were made up in phosphate buffer at pH 6, and varying numbers of tablets, capsules and measured amounts of oral suspensions were ground with distilled water and diluted in phosphate buffer to give high and low average final dilution figures. Insoluble benzathine penicillin G and V were dissolved in fresh formamide before dilution. Subsiduary standards were supplied from the Commonwealth Serum Laboratories as penicillin G potassium with a potency of 1600 international units per milligram, penicillin V acid of 1661 I.U. per milligram, and phenethicillin potassium of 1004 I.U. per milligram (1411 I.U. by iodometric

chemical assay with 60% of L isomer, complying with American F.D.A. limits and the approximate percentage of this more active component in the products available). Beecham Research Laboratories provided standard ampicillin and cloxacillin.

Consistent results were difficult to obtain, because slight variations in the weighing of standards and the pipetting of dilutions produced minor errors. This, no doubt, is one reason why some laboratories use a standard curve procedure with Bacillus cereus spore plates. Nevertheless, most of the products tested averaged, over several tests, approximately 100% of the stated dose, with a range of 80-120% in a few cases (Table VII). Stability tests were also conducted on tablets and capsules after 6 months unsealed storage at room temperature, with no apparent loss in any of the prepared dosage forms.

Table VII. Average Percentage of Potencies Claimed. Before and After Storage.

	Before	After
Penicillin G		
benzathine (200,000^{u} tablet Wyeth)	105	105
benzathine (200,000^{u} tablet C.S.L.[1])	100	-
crystalline[2] (200,000^{u} tablet Glaxo)	100	95
crystalline (400,000^{u} tablet Glaxo)	100	-
Penicillin V		
potassium (125 mgm. tablet Sigma)	100	100
potassium (125 mgm. film tablet Abbott)	100	100
potassium (250 mgm. capsule Faulding)	105	105
potassium (125 mgm. capsule C.S.L.)	105	-
calcium (250 mgm. tablet C.S.L.) over 2 years old	80	80
calcium (125 mgm./3.5 ml. oil suspension Boots)	120	-
benzathine (125 mgm./5 ml. aqueous suspension Sigma)	100	-
Phenethicillin potassium (250 mgm. tablet Sigma)	100	100
Ampicillin acid (250 mgm. capsule B.R.L.[3])	95	-
Cloxacillin sodium (250 mgm. capsule B.R.L.)	110	-

1 Commonwealth Serum Laboratories

2 Crystalline - not stated whether potassium or sodium salt

3 Beecham Research Laboratories

CHAPTER VI.

PENICILLIN ASSAYS AND SENSITIVITY TESTS.

Assays of Body Fluids.

Because of the heterogeneous composition of some penicillins, microbiological assays have been widely employed in this type of work, the total biological activity being more important than the total antibiotic calculated from chemical titrations using the iodometric method. Microbiological assays consist of tube dilution and plate diffusion tests, and the many other systems listed by Florey et al. (1949). The body fluids include serum or plasma, cerebro-spinal fluid, urine and various exudates. These are assayed as such or diluted in similar fluids with standard controls. Urine penicillin is sometimes extracted into solvents, particularly if the urine is contaminated, and then taken back into buffers for actual assay (Bond et al. 1963). Test specimens for the experiments described in this thesis were kept at -20°C. and assayed in batches.

The tube dilution method is usually based on doubling dilutions of the penicillin test fluid in tubes of suitable nutrient broth at pH 7, arithmetical progressions sometimes being interpolated between certain tubes to obtain a more accurate end-point. For the penicillins, the test organism is commonly a standard sensitive Staphylococcus aureus of the

Oxford strain (NCTC 6571), and this is added to $\frac{1}{2}$ - 5 ml. of final broth and penicillin mixture in each tube as 0.02 ml. of an undiluted or a 1/100 diluted overnight broth culture, or an equal volume of a 1/1000 dilution. After overnight incubation at 37°C., both the test and standard penicillin control rows are read at the last tube showing either no growth or estimated 50% growth. From the end-points the unknown assay is then calculated from the sensitivity of the test organism to the specific standard penicillin.

The method is dependent on sterile specimens and is obviously not very accurate, and consequently its use should be confined to sterile specimens anticipated to contain high levels of penicillin. Serum additions of 25-95% to the nutrient broth have usually resulted in lower readings, but if these are read against standards diluted in similar serum, reasonable total penicillin results may be obtained. The main advantage of serum with or without phenol red as a pH indicator is an increase in growth and an easier definition of the end point. The disadvantage is the increased destruction of antibiotic with overnight incubation.

For low level, accurate assays, the plate diffusion method, measuring inhibition zones against a standard curve, is the only acceptable technique and it is utilised for dose/response curves with all penicillins. Florey et al. (1949) list Staphylococcus aureus, Bacillus subtilis, Sarcina lutea

and many other organisms as suitable for this form of assay.

A Modified Assay Technique.

In this study the standard method of Grove and Randall (1955), has been modified using S. lutea seeded on to an agar base layer. The assay is capable of measuring levels below 0.01 units/ml. of penicillin G, and less than 0.03 micrograms of penicillin V, after overnight incubation at 26-28°C. The modifications have consisted of cups cut out of the agar instead of steel cylinders, or paper discs, and a reduction in the standard curve after trials on a full 6-point assay. The plastic petri dishes used were of standard internal measurements, with quite flat and regular surfaces permitting reasonably accurate assays from 4, 3 and finally 2 point standard curves, at 0.1 and 0.5 units (or micrograms) per ml., using 2 cups for each point and unknown. Single batch seeded agar was used for each series of tests, and a composite 3-4 point standard curve was made for reference each time a new seed suspension was prepared. For serum assays, sterile serum at pH 7.4 was used for standard dilutions, or 2-3% bovine albumin (fraction V) at similar pH. This amount of albumin with similar binding capacity has proved satisfactory with all the penicillins except cloxacillin, and this has required 5% albumin for a comparative assay in the system employed. Ampicillin was quite satisfactory at 3%, although

Knudsen et al. (1961) recommended 4% albumin.

Limits of error were calculated by Dr. J. R. Coulter as ± 18% for penicillin G plotted as a standard 3 point curve on semilogarithmic paper from 6 pairs of 2 randomised inhibition zones for each point. The average error only amounted to ± 5%, and repeated tests have shown that, at least for clinical and individual purposes, a 2 cup reading against a 3 point standard curve without a correction point is adequate and more accurate than tube assays.

Bunn et al. (1960) discussed the marked differences found in the standard serum assays of penicillin G, V and phenethicillin, noting that cup-plate assays were generally higher than parallel serial tube dilution tests and that a serum diluent gave the most consistent results. They found 1.75% bovine albumin a reasonably suitable substitute for serum, but they thought that assay comparisons of different penicillins were invalid on account of the wide discrepancies with different assay procedures and with identical assays performed in different laboratories. Apparently they were not aware of the fact that they were measuring total serum penicillin and that varying serum binding would not alter the total assay figures given from specific standard curves. The variable total assays were, however, probably invalid on account of differences in standard weighing and dilution, and also because of different isomer contents of test and standard

products. Lack of adequate pH control may well have been another important factor.

There has been definite confusion in some quarters concerning the identity of the substance actually measured in standard tube and plate assays, some thinking that it is merely unbound, free penicillin. However, it is clear that only a total figure can be measured against a total standard curve. Free penicillin levels are derived from calculations involving known serum binding percentages and measured total levels (Smith et al. 1956), and from direct measurements after removing serum protein and bound penicillin, the latter having been studied at therapeutic levels by forced filtration through dialysis membranes (Bond et al. 1963).

Assay Expression.

Early assay results were expressed in dilutions of amorphous crude penicillin, but this was quickly replaced by the Florey or Oxford unit. This was approximately equal to the present International unit of penicillin G, which is the biological activity contained in 0.6 micrograms of the International Penicillin Standard held at the National Institute for Medical Research, London (pure crystalline penicillin G sodium). With the advent of penicillin V, assays were still expressed in international units (I.U.) relative to penicillin G, but the later semi-synthetic penicillins resulted in the adoption of a standard based on weight of specific penicillin

per unit volume of test fluid (Garrod 1960a). This has come about because the penicillins show varying activities against different test organisms.

Penicillin G is still expressed by many in units, because there is an international standard but, as all the other penicillins are now quoted in micrograms/ml., specific standards must be obtained as close in content as possible to the product administered. (W.H.O. recommendations for fully categorised standards 1964). Those used in this thesis are listed under potency tests in Chapter V. Bond (1964) suggested that arbitrary units of activity be assigned to specific standard penicillins for formal assays, because of small amounts of differently active penicillin metabolites detected in chromatographic studies of blood and urine. These invalidate the accurate assay of specific weight of material, but clinical assays would not seem to warrant this system.

Serum Effects on Assays.

Serum binding has been generally accepted as the cause of the usual but not invariable serum inhibition of penicillin seen in laboratory assays, and Eagle and Tucker (1948) have shown that increasing percentages of serum exponentially inhibit activity in tube tests, while Smith et al. (1956) discovered an apparently similar effect in plate assays, with penicillin V being impaired more than G. The report of Rammelkamp and Keefer (1943b) on the serum enhancement of pen-

icillin activity is, however, brought to mind when tests conducted by me with Dr. J. R. Coulter are considered. These show that while some staphylococci are protected by serum, others are definitely killed more quickly in benzyl-penicillin and serum than in penicillin and broth.

Six-hour viable counts were used with fresh and heated serum obtained from several donors. Growth rate tests in serum without added penicillin have shown a test haemolytic streptococcus not to be affected by 30% serum (Tompsett et al. 1947), and Velu and Chabanas (1947) reported serum favourable to some bacteria and unfavourable to others. In our experience staphylococci have been variously inhibited by up to 100% serum. It is therefore necessary to recognise the direct but variable effect of serum on bacteria, although 18 hour tube dilution assays, using staphylococci killed more rapidly in penicillin and serum at 6 hours, show the usual reduced activity with added serum. Presumably at 6 hours there is more killing of these staphylococci in penicillin and serum than in broth, but at 18 hours visible growth is seen in the serum tubes at anticipated reduced activity levels.

In our work 2 ml. lots of serum, nutrient broth and alternate 20% mixtures of broth and serum were separately inoculated with overnight successive cultures of the Oxford, Wood 46 and V.W. strains of coagulase positive staphylococci.

All three organisms showed a minimum inhibitory concentration of 0.02 micrograms per ml. for penicillin G in broth, and the inoculum gave a starting count of 10^{5-6} organisms per ml. Freshly prepared penicillin was added in 0.04 ml. quantities of varying concentration. After incubation from 0-6 hours at 37°C. a growth curve was constructed by the techniques of Miles et al. (1938), from viable counts taken at ½-1 hour intervals.

Representative curves are shown in Figures 4, 5 and 6. The lag phase of bacterial growth was shortened as the concentration of penicillin increased, and the proportion of survivors at 6 hours decreased. There was, in addition, a limiting concentration of penicillin above which there was no change in the shape of the curve.

Growth rates in serum were usually slower than in broth, but penicillin killing rates varied considerably. A maximum killing effect was achieved with 0.13 ug./ml. of penicillin for the Oxford strain in broth, and just a little more in serum. The Wood 46 and V.W. strains gave maximum killing at 0.08 ug./ml. in broth and 0.3 ug./ml. in serum. Our test sera were definitely inhibitory to the growth and killing of Oxford staphylococcus and an attempt was made to elucidate this action by testing various mixtures of broth and serum. In fact similar curves were plotted with a slope intermediate between that of serum and broth, both with and without penicillin, for both 20% broth 80% serum and 80% broth 20% serum (Figure

5). Quinn (1964) found, however, that all sera did not inhibit all staphylococci and streptococci, and tests of our other staphylococci in the absence of penicillin showed a variable inhibition of growth.

The possible effect of serum inhibitors of penicillin itself was largely controlled by keeping the test time down to 6 hours at 37°C. Serum binding of penicillin was not thought to be the cause of inhibition in our opinion, because of the observations that V.W. and Wood 46 strains were actually killed more quickly in serum-penicillin than in broth-penicillin. This was felt to be a direct effect of serum on a particular organism, as it was noted that our different test sera behaved similarly with each strain. The conclusion is that direct effects of inhibition or enhancement of killing probably explain much of the variation in tube tests.

The explanation then of serum-inhibited tube tests is, in our opinion, not due to serum binding, because significantly more penicillin is not dissociated or available in broth dilutions compared with serum, nor is there any measurable binding of penicillin G at the 37°C, under the conditions of testing (see pages 75-79). In spite of opinions to the contrary (Tompsett et al. 1947), the inactivation rates of penicillin in serum fit in well with an observed one tube drop in activity, so that increased destruction with an added direct effect of serum on the organism becomes an acceptable

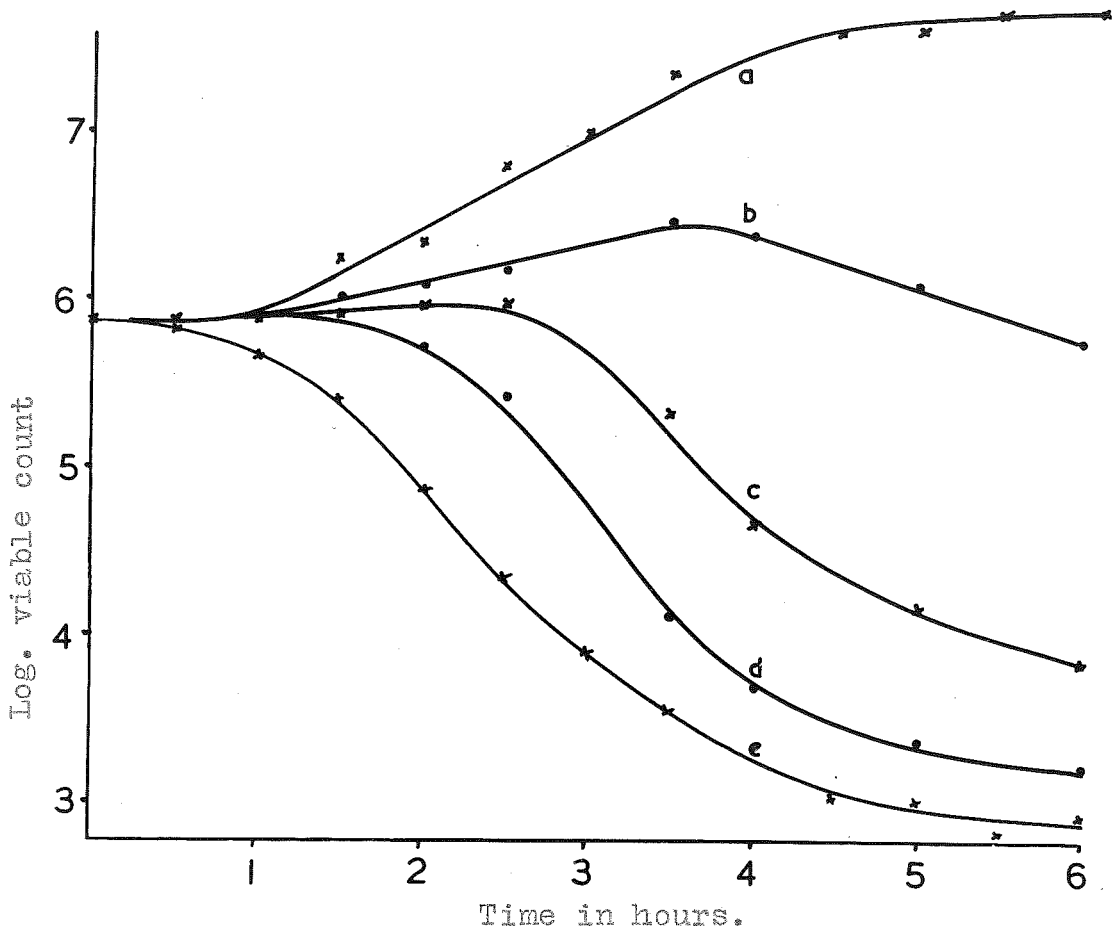


Figure 4. Viable counts of Oxford staphylococcus in broth with and without penicillin G.

(a) broth
(b) 0.006 ug./ml. penicillin G
(c) 0.012 ug./ml. "
(d) 0.08 ug./ml. "
(e) 0.13 ug./ml. "

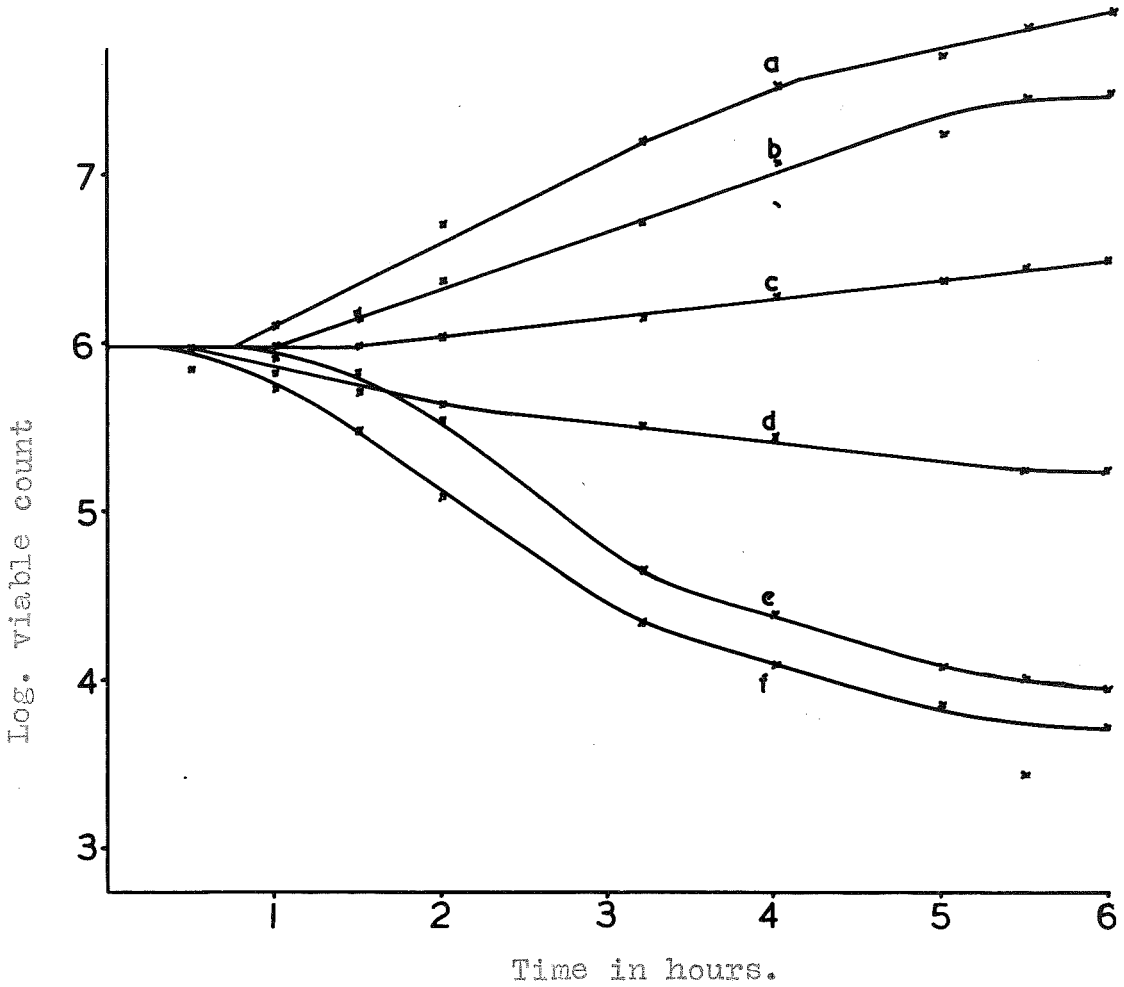


Figure 5. Viable counts of Oxford Staphylococcus in serum and broth with and without penicillin G.

(a) 100% broth
(b) 20% broth
(c) 100% serum
(d) 100% serum with 0.15 ug./ml. pen.G
(e) 20% broth with 0.15 ug./ml. "
(f) 100% broth with 0.15 ug./ml. "

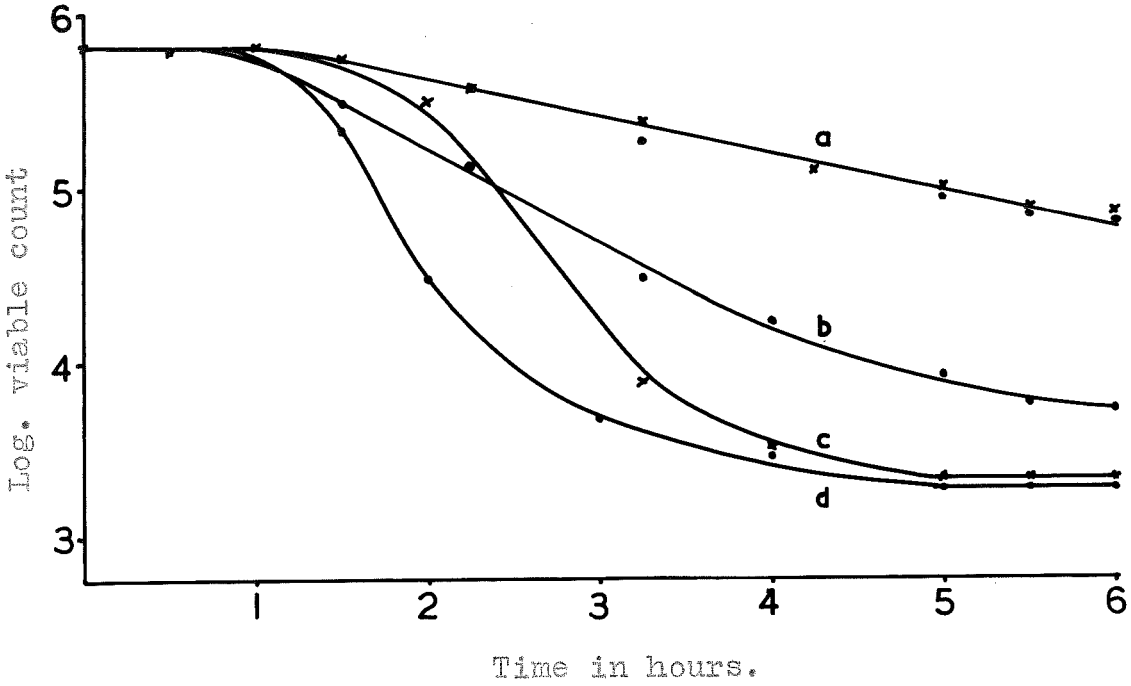


Figure 6. Viable counts of Staphylococcal V.W. and Wood 46 strains with penicillin G in broth and serum.

(a) 0.04 ug./ml. in broth

(b) 0.15 ug./ml. in broth

(c) 0.15 ug./ml. in serum

(d) 0.3 ug./ml. or higher in serum

explanation (see pages 65-67). The difficulties of individual variations in sera are explained by the large increments in doubling dilutions, and destruction rates appear to incidentally parallel binding figures.

When the serum-penicillin sensitive staphylococci are seeded in agar plates, serum impairment of inhibition zones is also demonstrated. I conducted some experiments with cellophane sacs which indicated that serum binding is in this instance the probable explanation of this phenomenon, plus some serum interference with the agar diffusion of free penicillin.

Cellophane sacs in cylinders were inserted into agar cups cut from Sarcina lutea seeded plates, and both penicillin G and phenethicillin in serum gave good but impaired zones compared with buffer tests, and smaller zones compared with plain agar cups, the inoculation being at room temperature and the incubation at 26°C. Non-permeable serum obviously has no direct effect on the growing edge of the organism, and full zones developing with penicillin G and phenethicillin in serum after only 15-30 minutes of permitted diffusion also rule out the effect of penicillin destruction over the remaining 18 hours incubation. Phenethicillin was inhibited by serum to a greater extent than penicillin G in both agar and cellophane cylinders, and this appears to be the result of a lowered free penicillin diffusion gradient from the more

highly bound reservoir, even with rapid dissociation rates. Cellophane membrane retardation of penicillin diffusion would finally explain the overall smaller cellophane cylinder zones compared with plain agar cups. Bovine albumin is known to reduce plate inhibition zones to the same extent as serum, and as penicillin binds to the albumin fraction, this is no doubt also only a binding effect.

Comparative Assays.

The use of the same assay methods for all penicillins has been of great assistance in obtaining blood level curves, but the assessment of comparative levels has led to much fruitless argument. Rinsler and Cunliffe (1956) mentioned the difficulty of comparing blood levels of penicillin G and V because of the greater biological activity of G against S. lutea compared with penicillin V. Previous workers such as Putman et al. (1955), while using either G or V as a standard, correlated G and V levels on their respective potencies in units. Rinsler remarked that "it is impossible to make a really valid assay of one penicillin in terms of another". He points out, however, that although "we directly assay penicillin G in terms of an international standard preparation of G expressed in units, and V in terms of a reference standard of V by weight, the potency of the two penicillins may be indirectly related on the assumption that 1 mg. of V has an activity close to 1700^{u} of G". (Our reference standard pen-

icillin V has 1661^{u} of activity). This was taken further by Williamson et al. (1961) who attempted to relate three assays in units (as well as by weight of specific penicillin), but Bond et al. (1963) rejected the usefulness of this.

McCarthy and Finland (1960) unsuccessfully advocated an indication of the content by reference to the weight of molecular equivalents of the specific penicillin acids, and they also used penicillin activity equivalents relative to 1 microgram of penicillin G in comparing equivalent doses of the phenoxypenicillins. The latter, like relative units, are only meaningful for the particular organism under test, and principles of biological standardisation would also be contravened. These require, among other things, that the active substance assayed be the same as the active substance in the standard (Miles 1952; World Health Organisation 1954, Technical Report Series No. 86).

Mathews (1960) has discussed the interpretation of antibiotic blood level curves based on the common 1, 2, 4 and 6 hour venous blood samples, and he has cited the usual lack of planning for statistical analysis and lack of sufficiently standardised test conditions. Contradictory conclusions reached by many different workers were noted and various attempts were made to improve standardisation. Latin square designs were designed to randomise the inoculation of agar plates, and Mathews himself suggested plotting curves from samples randomly taken at allotted times from a large

number of trial volunteers. This resulted in the elimination of local variations in the assay system and it has given outer curve ranges instead of one composite curve approximating to the decay of penicillin in the blood. This was thought of as providing more information relating to the levels obtained with specific penicillins.

Single therapeutic and comparative parameters were also proposed, such as higher peak blood levels and bigger "areas under the curves" with the phenoxypenicillins. Mathews rejected these, claiming that there was no knowledge of the relative importance of high intermittent peak and low persistent levels. Bearing in mind the fact that none of the oral penicillins produce persistent levels (low or high) and that satisfactory clinical responses are usually obtained, the peak level parameter and to a lesser extent the "area under the curve" would seem to be reasonable points of assessment.

Too fastidious a handling of clinical assays, even on a comparative basis, does not seem warranted, except for the obvious control of such simple but important factors as assay organism, type of agar medium or broth, identical diluting fluids and specific standards. Bond (1964) showed that more representative total penicillin levels resulted from the use of homologous serum as the standard diluent in serum assays, but she did permit pooled serum as a practical alternative. It also seems that 2-5% albumin is a reasonable substitute,

at least in clinical laboratories, since it provides a similar binding capacity to serum.

Hewitt (1963) reviewed the laboratory comparison of different penicillins, and in assessing the relative activities of the penicillins *in vitro*, he tabulated the useful procedures as being bacterial sensitivity (minimum inhibitory concentrations) and antimicrobial serum activity tests (serum inhibitory titres). The latter test depends on selected pathogenic bacteria instead of the common non-pathogens used so frequently in serum level assays, and it was introduced as a tube dilution test originally to provide guidance in effective antibiotic therapy for resistant streptococcal subacute bacterial endocarditis (Schlichter *et al*. 1949). Many have since used it for comparing the activity curves of the phenoxypenicillins in healthy volunteers (Jones and Finland 1955, McCarthy and Finland 1960, Griffith 1960 and Colville and Quinn 1962), and they have claimed that equal doses of penicillin V and phenethicillin produce comparable antibacterial activity, which is two or more times greater than that produced by the same dose of penicillin G; the latter requiring a double dose to equalise the effect. The validity of the test has recently been questioned by Knudsen (1964), who thinks that broth dilutions, with consequent apparent dissociation of bound penicillin, do not give a true picture of serum activity *in vivo*. Serum or broth dilutions dissociate

the penicillin, but overnight incubation and subsequent penicillin inactivation would counteract the invalid results to a variable extent.

In conclusion, the evidence points to the fact that with constant techniques and a good knowledge of bacterial sensitivities and penicillin pharmacology, peak total levels as assayed at present, together with activity titres in broth, do give a reasonable progressive guide both to the degree of bacteriostatic effectiveness *in vivo* for individual situations, and for the comparative assessment of new penicillins under normal clinical conditions. A series of my total penicillin serum assays, taken from actual patients, is presented in Chapter VIII.

Penicillin Sensitivity Tests.

The rapid diagnostic method uses impregnated paper discs, which are placed on agar plates seeded with the organism under test (Stokes 1960). A convenient specific method for laboratory study is the serial doubling dilution system applied to single or combined antibiotics in suitable nutrient media, either as liquid in tubes or solid agar in tubes or plates. Each dilution, including a control containing no penicillin, is inoculated with an equal amount of the pure organism whose penicillin sensitivity is being tested. The dilutions are then incubated overnight at 37°C. and read as the last tube or plate showing no growth (Florey *et al.* 1949,

and Grove and Randall 1955).

The tube test is conducted with ½ - 1 ml. volumes at pH 7.4, and the inoculum is usually 0.02 ml. of a pure or 1/500 diluted overnight broth culture of the organism, the size of the inoculum not making a significant difference to the end-point in broth. A pH indicator such as phenol red is often added to the tube test, so that a colormetric end-point can be read in media containing glucose. Pooled serum in a concentration of 25% or greater is commonly included to improve the growth of fastidious organisms, and to reduce the sensitivity figure for clinical assessment (Stokes 1960, and Bond et al. 1963).

In sensitivity tests, serum inhibition has again been attributed to serum binding. We believe that the multiplying organism in the dilution showing the first growth has so many new penicillin combining-sites that a progressive, rapid dissociation must occur, keeping a constant between bound and unbound penicillin. Thus no penicillin should be left in the bound state to influence the final titre. Bond et al. (1963) showed decreased sensitivities with the progressively bound phenoxypenicillins in titrations performed in serum compared with broth, but apart from the very highly bound phenbenicillin and cloxacillin where the binding is probably critical enough to effect dissociation rates, the variable 4-8 fold decreases in sensitivity with serum are probably not due

to binding but to relative increases in penicillin destruction over 18 hours. To a lesser extent the varying direct effects of the penicillins and sera on different organisms may also play a part (see pages 102-109). It is also of interest to note that instability and the production of degradation factors have recently been verified as decreasing the sensitivity of cephalothin between 12-24 hours (Wick 1964).

These tests permit only a bacteriostatic measure of a drug's anti-bacterial activity, and this is expressed as a minimum inhibitory concentration (M.I.C.) in units or micrograms per ml. for each specific organism and antibiotic. Bactericidal information requires an extension of the test by some form of subculturing or viable count performed on the dilutions showing no growth at different times of incubation.

The activity of the available penicillins against common pathogenic organisms is listed from previously published sources in Table VIII for the benzyl and phenoxypenicillins, and Table IX for the new penicillins. Only broth tests are reported to exclude penicillin inactivation as much as possible. Penicillin V is seen to be the best of the phenoxy group, being almost as effective as penicillin G against Gram positive cocci, with phenethicillin close behind. Propicillin and phenbenicillin progressively diminish in activity against staphylococci but not streptococci, as the serum binding increases (Bond et al. 1963). Correlation of the higher pot-

ency of penicillin G with the higher blood levels of the phenoxypenicillins (see Table II and Figure 1 on pages 60 and 61) shows penicillin V and phenethicillin to be approximately equal in efficiency, with penicillin G somewhat less so because of its much poorer oral absorption weight for weight, and more variable serum levels following food.

Table VIII. Published Average Minimum Inhibitory Concentration in Broth. *

Expressed as Micrograms per ml.

	Penicillin G.	Penicillin V.	Phenethicillin
Staph. aureus (sensitive)	.02	.03	.03
Str. pyogenes	<.02	.02	.03
Str. pneumoniae	.02	.02	.06
N. gonorrhoeae	.02	.03	.12
N. meningitidis	.03	.12	-
Cl. welchii	.06	-	-
Str. faecalis	2	4	4
H. influenzae	1	4	4
Salmonella Spp.	8	128	>250
E. coli	64	128	>250

* (Gourevitch et al. 1959, Rolinson and Stevens 1961, Barber and Waterworth 1962, and Bond et al. 1963).

Table IX. Published Average Minimum Inhibitory Concentrations in Broth. *

Expressed as Micrograms per ml.

	Ampicillin	Cloxacillin
Staph. aureus (sensitive)	.05	.15
Staph. aureus (resistant)	-	.4
Str. pyogenes	.02	.07
Str. pneumoniae	.02	.25
N. gonorrhoeae	.12	.5
N. meningitidis	.06	.5
Str. faecalis	2	25
H. influenzae	.25	16
Salmonella Spp.	2	-
E. coli	6	-
Proteus mirabilis	4	-
Proteus vulgaris	64	-

* (Rolinson and Stevens, 1961, Barber and Waterworth 1962, Knudsen et al. 1962).

I have conducted many clinical and experimental bacterial sensitivity and serum activity tests over the course of the past four years in our laboratories. Complete records were not kept, but they were consistent with the published figures above.

CHAPTER VII.

CLINICAL DISCUSSIONS.

In vivo Application of Laboratory Tests.

This chapter concerns the relative importance of total and free penicillin levels, and their correlation with bacterial sensitivities to penicillin estimated in 25-95% serum or nutrient broth. Apparently good correlation of the serum inhibition of penicillin sensitivity with degrees of protein binding has been noted by Bond et al. (1963), who suggest that only the free active portion of the penicillin influences the result. Knudsen (1964) claims that free levels should be assayed in relation to M.I.C.'s in broth, and that if total levels are required they should be assayed in relation to M.I.C.'s in serum.

The work of Dr. J. R. Coulter and myself on in vitro serum effects and my own studies on penicillin inactivation seem to prove, however, that increased penicillin destruction and the serum inhibition of test organisms is the cause of the serum effect observed in tube titrations. In addition, the rapid dissociation of loosely bound penicillin coupled with bacterial growth and consequent antibiotic take-up by new combining sites, would seem to result in the total penicillin being ultimately available in those tubes showing

growth in the serum system. A convenient measure of the M.I.C. of an organism at 6 hours with or without serum would be useful to eliminate the error introduced by penicillin destruction during the 18 hour test, in both serum and broth (see Table IV), and it would give a more valid in vivo assessment of antibiotic requirements.

This has, in fact, been performed by us by estimating staphylococcal killing rates at 6 hours as depicted in Figures 4-6. Concentrations of penicillin G in broth approximating to M.I.C. broth figures, show some definite killing, while concentrations 4-10 times the M.I.C. show a maximum killing effect, this varying with the organism under test. The M.I.C. in serum and the relative killing rate with various concentrations of penicillin in serum sometimes show a similar relationship to broth tests, but some staphylococci exhibit increased sensitivity to penicillin in serum compared with penicillin in broth. Overall, it would seem that the simple 18 hour M.I.C. in broth, with the smaller amount of penicillin destruction, is still a worthwhile test in arriving at an in vivo assessment, while the M.I.C. in serum is of dubious significance, even in cases of endocarditis and septicaemia.

With regard to total and free serum levels, a reasonable concept of the obviously dynamic equilibrium set up by a diffusing antibiotic must first be worked out and then applied. Free unassociated penicillin seems to be the only

portion available for the first free diffusion of penicillin from the blood and the first take-up by bacteria or tissue protein. An estimate of free penicillin can prove early absorption levels to be above observed M.I.C.'s in broth, and this has been shown to be the case for sensitive bacteria and normal doses of the phenoxypenicillins (Bond et al. 1963).

Some believe that the portion available in the tissues is dependent upon the free fraction present in the serum at any given moment, because diffusion is free and rapid (Knudsen 1964). However, rapid dissociation of loosely bound penicillin must follow diffusing free penicillin, and this means that a large portion of the total amount in the serum bound reservoir, at any one time, becomes quickly available to maintain the "free-bound" constant and equilibrium, in lymph and a limitless tissue area (see "Lymph and Tissue Binding", page 73). In addition, our dialysis tests indicate much lower binding figures for most of the penicillins at a strictly controlled physiological pH. Free assays from negative pressure filtration studies are, as yet, not really valid owing to lack of sufficient control of numerous parameters, which include pH effects consequent upon loss of carbon dioxide from serum test samples.

Valid tissue levels are, to date, impossible to measure (see page 83), and the conclusion is that routine total assays of penicillins in actual use give reasonable in vivo

assessments, free levels being unnecessary. The more direct serum inhibitory titres tested with specific pathogens in broth will always keep their place in the form of comparative antibiotic assessments for serious systemic infections, and for the comparison of new penicillins. Finally, 6 hour viable counts of specifically isolated pathogens in serum may effectively be added to the present serum inhibitory titres in unresponsive cases.

The literature expresses doubt concerning the question of intermittent versus continuous antibiotic levels, and laboratory investigation of this problem will probably never solve it. Indirect evidence is, however, available from viable count tests, where a lag period of 3-4 hours is found before the multiplication or killing of bacteria commences, in the presence of very small amounts of penicillin. When this lag interval of bacteriostasis is added to the 4-6 hourly dosage schedule covering the rapid excretion of penicillin, the period of sub-inhibitory or zero blood level with intermittent oral therapy appears to be covered.

Clinical experience has also dispelled doubts regarding intermittent dosage with the observation of good responses in moderate and antibiotic-accessible infections. On the other hand, serious bacterial diseases difficult of access, such as subacute bacterial endocarditis, osteomyelitis, suppurative tenosynovitis, pyelonephritis and brain abscess are

known to relapse on such therapy, and it is obvious that a continuous high blood level is necessary to achieve the effective penetration of bactericidal levels of antibiotic, or at least to maintain a bacteriostatic concentration until body macrophages can deal with the remaining metabolic persisters. Laboratory studies of staphylococci seeded in blood clots have confirmed that under these circumstances and with these particular organisms, persisters from intermittent exposure can be finally eliminated by maintaining good concentrations of penicillin over a period of several days. (Editorial J.A.M.A. 1961).

Inconsistent clinical responses in relation to laboratory sensitivity reports do occur, but a careful investigation more often than not reveals the reason. Ampicillin frequently gives a satisfactory clinical response even though resistance has been reported from disc sensitivity tests. The likely factors involved may be inadequate disc concentrations, or body defences which are alone sufficient to control the infection. The reporting of a commensal instead of the real pathogen, cross-infection with another organism, or different growth and metabolic characteristics in vivo compared with in vitro systems may be additional sources of error.

Sometimes lack of clinical response is noted where full sensitivity has been reported, and the factors operating here may be irregular absorption, abnormal tissue penetration, as

with abscesses and suppurative tonsillitis, or the appearance of resistant mutants. In addition, the presence of debilitating disease, particularly when the blood levels and M.I.C. are dangerously close, may also result in an unsatisfactory clinical response.

Special Clinical Conditions.

Endocarditis:

A survey of the history of oral penicillin therapy reveals that some physicians occasionally advocate prolonged treatment of subacute bacterial endocarditis, osteomyelitis and even meningitis, with various oral products substituting for or following on accepted parenteral regimes.

In the case of endocarditis, it is difficult to believe that bacteria, embedded within fibrotic vegetations, can receive a lethal exposure to penicillin from an intermittent diffusion gradient. The acellular structure of these vegetations indicates that macrophages cannot migrate into the infected areas to destroy surviving bacteria. Adequate bactericidal levels from parenteral therapy must therefore be maintained to ensure sterilisation of the value without the assistance of body defence mechanisms. Should intensive oral therapy be attempted, careful supervision is required at both clinical and laboratory levels. This may be the case in children, who often exhibit reduced tolerance to parenteral medication.

Note should be taken of other evidence suggesting that relapses are common during the oral treatment of streptococcal pharyngitis and the bronchopneumonia following measles, many of these cases being admitted to hospital for parenteral therapy. Mohler et al. (1955), reporting on numerous domiciliary visits, state that approximately 50% of patients receiving a 10-day course of oral penicillin for streptococcus eradication had, in fact, discontinued their treatment by the third day. Many others had little or no idea of the correct dosage schedule.

Oral penicillin prophylaxis following endocarditis would appear to be of dubious value. Frequent large doses, prescribed in an out-patient department, with or without probenecid, would not produce blood levels comparable to those achieved with primary parenteral courses. The penetration of penicillin would fall short of that obtained with effective parenteral treatment, allowing deep-seated organisms to survive and form a nucleus for relapse.

Dental prophylaxis:

Concerning the prophylactic cover of dental treatment in patients with damaged heart valves, Rammelkamp (1957) recommends oral penicillin four times a day. The National Heart Foundation of Australia stresses the maintenance of good oral hygiene, together with the use of penicillin for at least 3 days, commencing the day before any necessary procedure, preference being given for parenteral injections,

with high dosage oral penicillin being permitted below 14 years of age. Reports also indicate that some authorities have instituted long-term oral supression of streptococcal mouth flora, in an attempt to reduce intermittent bacteraemia capable of colonising damaged heart valves. Naiman and Barrow (1963) have, unfortunately, reported strains of Streptococcus viridans isolated from the gum margin and pharynx which were resistant to 2^{u} of penicillin per ml. These were recovered from patients receiving oral penicillin for the prophylaxis of rheumatic fever and recurrent streptococcal infections. It therefore seems unwise to provide cover for patients beyond late adolescence in either of these conditions although further long-term studies are required before a firm opinion can be expressed.

Chronic Abscesses:

In osteomyelitis and bone and brain abscess, even though the blood supply is restricted by bone or fibrous tissue barriers, one could expect macrophages to eventually remove the few organisms persisting after effective antibiotic therapy, particularly with surgical drainage. Even the bacteriostatic effect obtained from subsequent intermittent oral penicillin and probenecid would, in all probability, be worthwhile.

In follicular tonsillitis, the published reports of Wehrle et al. (1956), and personal clinical experience, have

repeatedly shown that parenteral therapy is often needed for the first few days to eradicate streptococci and prevent relapse. In the case of children, conscientious parents will usually ensure that adequate oral therapy is maintained for 7-10 days. Established otitis media, even in children, invariably requires injections at the outset, followed by a period of oral medication. Meningitis requires parenteral management, but ampicillin may be an exception (Ivler et al. 1963), with high oral doses giving more prolonged blood levels than intramuscular injections. Relapsing gonorrhoea, due to relatively penicillin resistant organisms, has no doubt largely resulted from inadequate oral therapy. The unstable type of patient so often involved in this situation is a bad subject for self-administered medication, so that some portion of the total dosage should be given by injection.

Bronchitis:

The long-term prophylaxis of bronchitis with tetracyclines has been reported by Robertson (1965) as producing an increase in the number of isolates of tetracycline resistant beta haemolytic streptococci in otitis media and wound infections. For this reason, a return to oral penicillin has been recommended, as resistant streptococci are so far almost non-existent. Reference to ampicillin in the treatment of respiratory disease can be found in the historical Chapter III on page 36.

Probenecid.

Reference to the historical chapters shows probenecid to be an effective agent for blocking the renal excretion of penicillin, giving blood levels 2-4 times the normal figure, which are prolonged for 2-3 hours. This effect will again depend upon strictly supervised prescribing and administration, and should probably be reserved for long-term infected in-patients on parenteral and oral follow-up treatment. It should also be remembered that patients with incipient renal failure do not require probenecid, and quite high constant levels can be maintained without fear of direct toxicity. Doubts have been expressed regarding the toxicity of probenecid, but it is of interest to note that Boger and Strickland (1955) found no deaths in thousands of patients receiving the drug, which produced no greater gastric irritation than the tetracyclines.

Combined Therapy.

Combined oral penicillin and sulphadimidine was used by Wheatley (1953), to treat acute otitis media, and his review claimed that this combination produced the best results, which were also predicted by the laboratory. This was in spite of the fact that some have thought, on purely theoretical grounds, that sulphonamide bacteriostasis might inhibit the bactericidal effect of penicillin on multiplying organisms. The use of this oral combination is still pop-

ular for the general practice cover of pneumonia and its complications, even if side effects are probably increased. A synergistic effect of penicillin G or V with fucidin can be shown in laboratory tests with staphylococci, and this may be of use in methicillin and cloxacillin resistant infections. In general, however, combined antibiotic testing or therapy involving oral penicillins seems to have little place in the treatment of moderately severe infections.

Superinfections.

These include penicillin resistant staphylococci, pathogenic yeasts and Gram negative bacilli which may become established in parts of the respiratory, gastro-intestinal or female genital tract as secondary pathogens. This has been observed to some extent with all the penicillins but cloxacillin appears more liable to produce these complications (Stratford 1963). High 500 mgm. 6-hourly doses of phenethicillin in chest infections have been reported by Louria and Brayton (1963) to cause a troublesome increase in respiratory tract superinfections, when compared with the usual 250 mgm. doses.

Clinical Trials.

For some time, these have lacked adequate control but good clinical responses have given an indication of the degree to which pathogenic bacteria are exposed to penicillin in the tissues. The trials do not suggest any definite

preferences concerning the different oral penicillins such as penicillin V, phenethicillin and soluble penicillin G, because these different products have only been effective or otherwise under the conditions of the particular test.

However, a brief consideration of the history of oral trials shows that Flippin et al. (1951) conducted the first controlled experiment using different oral antibiotics under standard conditions of age and type of infection, plus a control series receiving a comparable dose of intramuscular penicillin G. Miller et al. (1954) used the siblings of children on oral penicillin for rheumatic fever prophylaxis as controls of the home environment, and in their statement of the efficacy of this prophylaxis they mention the difficulty of supervising oral medication. Weiss et al. (1959), reviewing the controversy over penicillin V and G, have concluded that, up to that time, most studies of the new penicillin V had not been adequately controlled, and that preference for penicillin V was not necessarily sound.

Many trials have found phenethicillin successful in a majority of moderately severe respiratory tract and skin infections, and each successive new oral penicillin has given acceptable results in partly controlled trials. These have included urinary and respiratory tract infections treated with ampicillin (Anderson et al. 1964), and penicillin resistant staphylococcal skin and wound infections treated with cloxacillin (Stratford 1963).

Trials of phenethicillin and penicillin V in general practice were conducted by Edgar (1960) and Schofield (1960), and in many paired infections a large percentage responded satisfactorily, with an apparently faster recovery rate with phenethicillin. Marshall (1960) did not confirm these findings but, as he concluded that at least 5 days therapy was needed, one suspects that his patients received the drugs for too short a time. Knudsen and Rolinson (1959) reported that phenethicillin blood levels were as high or higher than equivalent intramuscular penicillin G, but one must constantly remember the uncertainties of regular oral dosing, and accept the fact that parenteral penicillin should be given at least for the first 1-2 treatments of a fully established infection.

Trials of prophylactic penicillins continually appear from groups concerned with rheumatic fever prophylaxis and, although the oral penicillins are generally acceptable, long-term intramuscular benzathine penicillin G gives the best protection (Albam et al. 1964). Equivalent results in double blind trials covering various infections have been noted by Martin et al. (1963). These workers used parenteral penicillin G and oral penicillin V, with the criteria of efficacy being mortality, duration of therapy, duration and rate of decline of fever, and degree of leucocytosis. This form of trial did not permit patient or prescriber to know which drug was given, and other controls included an absence of patient

selection with randomising treatment assignments.

Lepper (1963) suggests more complete ecological studies of the penicillins, the pathogens and the host, to give a broader approach to experiments focussing on stated interrelationships, while attempting to control all other variables. Mathews (1960) has also suggested other methods of controlling laboratory tests (see page III), but one suspects that little additional information will result from these refinements, at least with regard to clinical assessments. Chapter VIII will present the results of assays performed on patients under widely varying conditions of hospital and clinical practice, and their correlation with clinical response and the types of penicillin used.

Penicillin Resistance.

Mutational types:

From a clinical point of view this appears to be almost entirely phenotypic, with naturally occurring penicillin resistant bacteria being environmentally selected as a group by the suppression of sensitive strains. The problem has been mostly confined to cross-resistance to the benzyl and phenoxypenicillins with strains of staphylococci which are natural penicillinase producers (Spink and Ferris 1947). Phage-typing, at least in some cases, has shown these bacteria to be substituted, by means of hospital cross-infection, for the sensitive strains usually found with normal flora in

an antibiotic-free environment (Rountree and Thomson 1949, and Chain 1962).

In the laboratory, gradual step-wise development of individual genotypic penicillin resistance, in sensitive staphylococci, has been repeatedly shown from *in vitro* tests with increasing sub-minimal inhibitory concentrations of all the penicillins, and these mutations have been found, almost invariably, not to produce penicillinase (Florey *et al*. 1949, Elek 1959, and Barber and Garrod 1963).

Penicillinase:

Penicillinase was first detected and defined as an enzyme by Abraham and Chain (1940). Many bacteria have since been found to produce this penicillin-beta-lactamase, and in staphylococcal penicillinase inactivation tests, both penicillin G and V are totally destroyed in one hour at 37°C., while phenethicillin, with a somewhat lower enzyme affinity, is more resistant with a loss of only 75% (Gourevitch *et al*. 1959).

Ampicillin is also very sensitive to penicillinase, and the production of the enzyme by certain strains of *E.coli*, *Proteus vulgaris* and *Aerobacter aerogenes*, appears to explain at least some of the ampicillin resistance in infections with these organisms, even though ampicillin is not always attacked by the penicillinases capable of destroying penicillin G and V (Rolinson and Stevens 1961, and Knox 1962). Cloxacil-

lin, like methicillin, is highly resistant to penicillinase apparently because of a protected nucleus (Knudsen et al. 1962). Staphylococci inherently resistant to cloxacillin and methicillin are only rarely found, and they are not expected to become an urgent problem (Barber 1962).

Penicillin amidases are also produced by some bacteria, but they do not seem to play any part in clinical resistance (Knox 1962). The new derivatives of the cephalosporins are also penicillinase resistant, but it is interesting to note that some staphylococci and Gram negative organisms are already known to produce a cephalothinase. (Third International Congress on Chemotherapy 1963).

Episomal Transfer:

Recent findings of episome-mediated multi-antibiotic resistance do not appear to be relevant to penicillin, except for ampicillin resistant Salmonella typhimurium raising the possibility of transfer to typhoid carriers (Editorial 1965, B.M.J.).

Penicillin Hypersensitivity.

Keefer (1951) and White(1953) provided some of the first reports of allergic reactions following the oral penicillin prophylaxis of venereal disease, but it was recognised that oral therapy was not as troublesome in this regard as previous parenteral treatments had been. Reports of mild reactions and occasional anaphylactic deaths have been noted

for all the penicillins (Barber and Garrod 1963).

The two types of reactions are classified as the serum sickness type with delayed urticaria or Id rash, fever and joint pains, and the anaphylactic type with immediate, profound shock (Hewitt 1963). How penicillin acts antigenically is not known, but the degradation product penicillenic acid appears to act as a haptene combined with protein (de Weck and Eisen 1960). The further derivative of this penicillin is penicilloyl, and as it has been thought by the latter workers to be less likely to sensitise, it has been conjugated with polylysine and used as a P.P.L. skin test with some good results. An indirect basophil degranulation test is claimed by Shelley (1963) to show a high proportion of hypersensitive individuals, the test being performed on serum samples.

Unfortunately neither serum nor skin tests show unequivocally that a patient is prone to anaphylaxis, although Brown and Moore (1964) claim that true and false negative P.P.L. skin test patients have shown no anaphylactic reactions in large numbers treated with penicillin. Penicillin skin sensitising and red blood cell agglutinating antibodies have been demonstrated, but there is no consistent correlation with the development of allergic disease, or necessarily with previous penicillin treatment (Josephson 1962).

The causes of sensitisation have usually been attributed to previous therapy, the use of topical applications,

"hidden" contact with milk and vaccines containing penicillin and possible skin fungi producing penicillin (Hewitt 1963). The penicillins all have the 6 A.P.A. nucleus and they are accepted as being cross-sensitising. The 7 A.C.A. cephalosporin derivatives may, however, offer a therapeutic alternative for the penicillin sensitive patient.

Asthmatics and patients with a history of other hypersensitive states are at risk from penicillin treatment, but those who have had previous anaphylactic reactions to the antibiotic present the most serious problem to the practitioner. However, Luten (1964) has reported that 8 patients with a history of anaphylactic reactions to penicillin G gave negative intradermal skin tests with 0.02 ml. of 50 or more micrograms per ml. of methicillin sodium, and furthermore, these cases tolerated methicillin therapy without experiencing untoward reactions. Despite this observation, the physician is probably wise to recommend an alternative antibiotic such as erythromycin or lincomycin (Jackson 1965), even though many such cases have, in an emergency, received penicillin without harm. An effective precaution, which may be taken when it is essential to administer penicillin, is to mix a dose of chlorpheniramine maleate with the antibiotic in the same syringe prior to injection. This is said to be more reliable than administering the antihistaminic orally or intramuscularly in a separate site. Adrenaline and soluble hydrocortisone should always be available when the possibil-

ity of penicillin hypersensitivity exists.

Fewer manifestations of allergy over the last year or two appear to be explained by a number of factors which include the control of penicillin residues in milk, less topical use of penicillin and greater advances in the techniques of purification of products for parenteral use.

CHAPTER VIII.

CLINICAL ASSAYS.

In view of previous comparative oral penicillin trials mostly employing volunteers on artificial dosage schedules, I obtained fresh blood samples from patients on penicillin therapy in private and hospital practice. It was my conviction that absorption of oral penicillins might be much lower in bed-ridden patients than in healthy students. Difficulties with regard to dosage, type of product, time of sampling and adequate clinical history were encountered, but these were overcome as far as possible. The number of individual assays amounted to 218, with some of these being repeats on the same patient.

Blood samples taken 1-5 hours after the dose were requested, and hospital specimens were, in the main, from patients who received penicillin before meals. The control of oral therapy in the patients of private practitioners was not possible, and a small percentage of negative assay results was probably due to sampling after the time of measurable levels, and not to a failure of absorption. Serum was separated as soon as possible on the same day, stored at -20°C., and batches of assays were performed every few days.

Specimens of serum for crystalline soluble penicillin G assay came from two sources. Firstly, I accumulated a few private patients receiving tablets of a low 200,000^{u} dosage

for bronchitis prophylaxis, and a small number of tonsillitis and streptococcal throat patients on tablets or capsules of 400,000^{u} for therapy. Secondly, a number of assays of soluble penicillin G were taken from a volunteer trial of film-coated tablets. These were compared with a larger number of insoluble benzathine penicillin assays from cases receiving moderately hard tablet streptococcal prophylaxis of rheumatic fever etc. in private practice.

Figure 7 on page 140 depicts the 200,000^{u} dosage as a comparison of individual and mean assays. The individual range is probably an expression of the many gastronomic variables, and the mean shows definite levels only over 4 hours. The benzathine figures are lower than those obtained with soluble penicillin G, the two being expressed in units, as both are salts of penicillin G or benzyl penicillin, for which there is an international unit. There is also no indication of the prolonged effect of oral benzathine inferred by some proprietary advertisements. Prolonged levels claimed under controlled circumstances by Welch et al. (1953) were only just measurable after 5 hours, and useless traces of the drug found up to 10-12 hours after the dose obviously represented the small amount of left-over insoluble salt hydrolysed to the absorbable soluble form in the lower small intestine. A few of the benzathine specimens produced negative assays, but as the majority on retesting assayed reasonable levels, late sampling was the probable explanation.

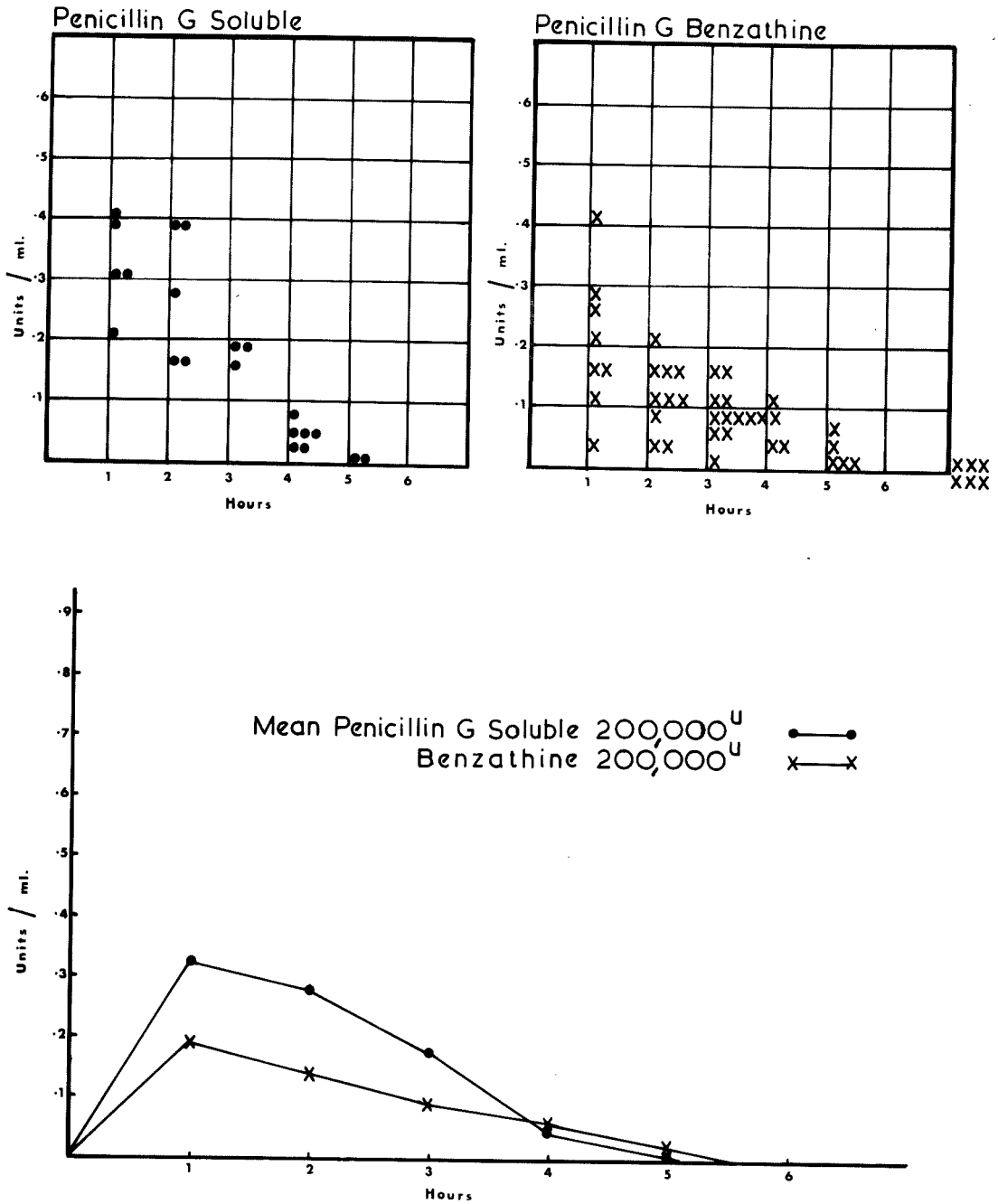


Figure 7. Individual and mean blood levels of penicillin G.

Table X on page 142 presents the penicillin G comparative blood levels for those cases from which a clinical response was available. The assays are again expressed in biological units, but approximate equivalents in micrograms weight are included in brackets (0.6 ug = 1^u, see page 101) for comparison with the phenoxypenicillins. Soluble and benzathine penicillin G are clearly acceptable prophylactic oral penicillins, and the soluble salt is probably acceptable as a therapeutic, even though the small number of cases presented do not permit of any real clinical conclusion.

The better absorbed penicillin V potassium and phenethicillin are also compared by individual and mean assays in Figure 8 on page 143. These were taken from hospital patients on therapeutic doses of 250 mgs. t.d.s. before meals, and when compared with Figure 1 on page 61, they are seen to confirm my predictions of generally lower levels than most previously published trials on volunteers. Phenethicillin peak levels in micrograms per ml. are about 1½ times greater than penicillin V and about four times as high as the equivalent dose and microgram level of penicillin G. Penicillin V produced a few negative assays compared with the fully satisfactory results for phenethicillin absorption, but on retesting they all gave measurable levels.

Higher doses of 500 mgs. were also assayed from a few cases of both phenoxypenicillins, some with and without pro-

Table X. Blood Levels and Clinical Responses.

Soluble and Benzathine Penicillin G.

Reason for Treatment	No. of Cases	Penicillin	Blood Level Range in Units/ml. Over 1-4 hours	Response
Streptococcal throat prophylaxis for rheumatic fever	18	Benzathine G 200,000^{u} b.i.d.	0.16 - 0.02^{u} (= 0.1 - 0.01 ug) 4 negative assays	No recorded relapses
Streptococcal throat prophylaxis for nephritis	3	Benzathine G 200,000^{u} b.i.d.	0.14 - 0.06^{u} (= 0.09 - 0.04 ug)	All relapsed when tablets left off
Chronic bronchitis prophylaxis	4	Benzathine G 200,000^{u} b.i.d.	0.28 - 0.06^{u} (= 0.18 - 0.04 ug)	1 repeatedly relapsed
Chronic bronchitis prophylaxis	2	Soluble G 200,000^{u} b.i.d.	0.3 - 0.1^{u} (= 0.2 - 0.06 ug)	Reasonably controlled
Tonsillitis	3	Soluble G 400,000^{u} t.d.s.	0.4 - 0.1^{u} (= 0.3 - 0.06 ug)	2 cured. 1 superinfection of tonsil with *E. coli*

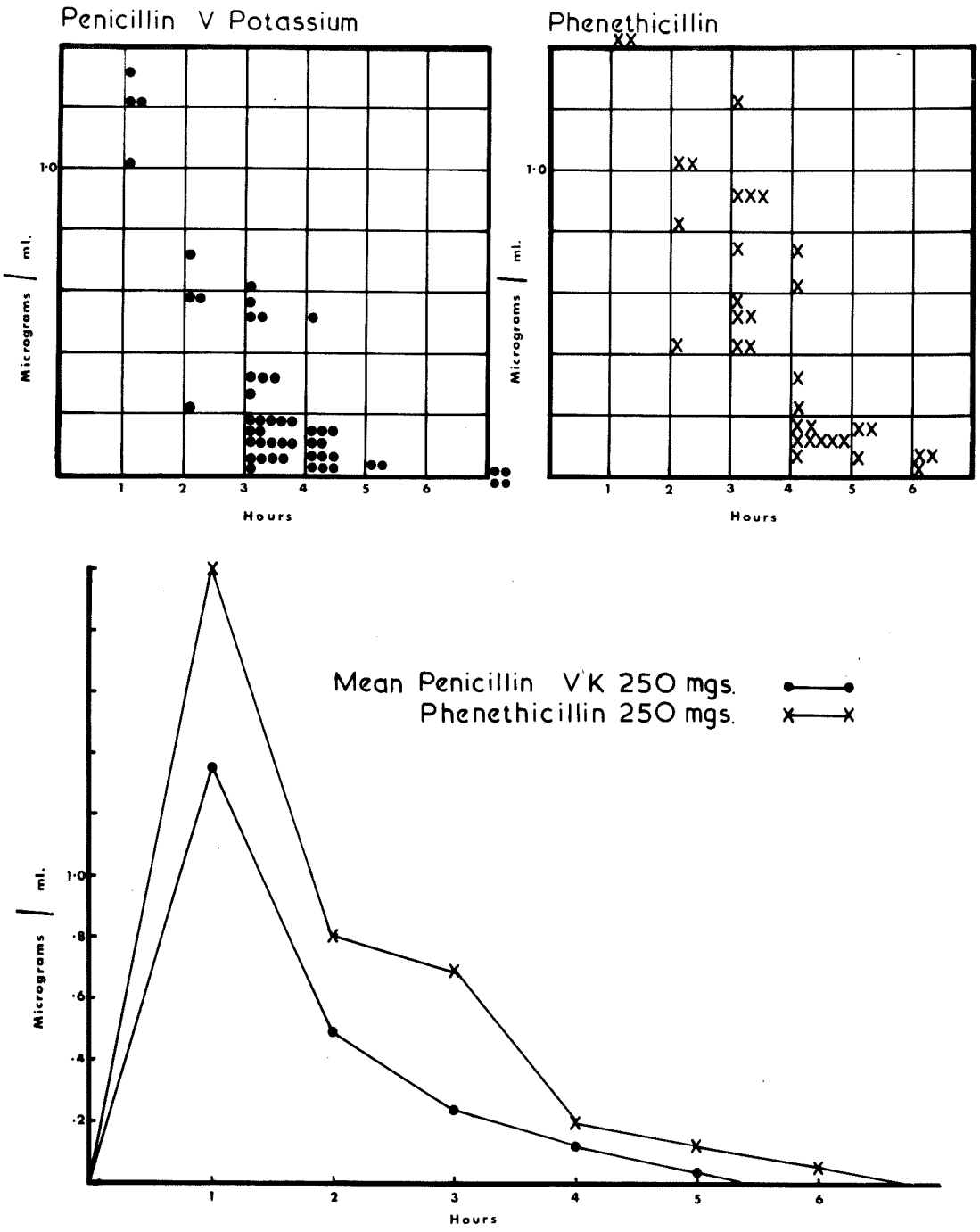


Figure 8. Individual and mean blood levels of phenoxypenicillin.

benecid as a kidney blocker of penicillin excretion. There was a definite raising and prolonging of blood levels, but this was variable and certainly not always a doubling up as often claimed for both probenecid and the higher doses. Occasional reports of therapeutic levels lasting beyond 5-6 hours on ordinary doses without probenecid probably indicate psychological delay of stomach emptying in volunteers apprehensive of venepuncture.

Table XI on pages 145 and 146 again compares the blood levels and available clinical responses. Definite levels of these phenoxypenicillins are comparable for an hour longer than for the penicillin G drugs, and the upper range is much higher. Both penicillin V and phenethicillin are shown to be worthwhile therapeutic agents in moderate streptococcal and penicillin-sensitive staphylococcal infections. This statement can be upheld, however, only if treatment is adequately supervised and persisted with for at least 5 days.

A few hospital patients were the source of some ampicillin and cloxacillin assays, and these are depicted in Figure 9, page 147, for 1 gram doses with and without probenecid, or 250 mgm. for ampicillin, and 500 mgm. for cloxacillin. The levels obtained confirm previously published averages as seen in Figure 1 on page 61, but probenecid did not significantly raise early levels of oral ampicillin under these particular conditions, even though some prolongation of later

Table XI. Blood Levels and Clinical Responses.

Penicillin V and Phenethicillin.

Reason for Treatment	No. of cases	Penicillin and dose	Blood Level Range in Micrograms/ml. over 1-5 hours	Response
Rheumatic fever prophylaxis	10	Penicillin V 250 mgm. b.i.d.	0.6 - 0.04 ug. 2 negative assays	1 relapse from irregular medication
Subacute bacterial endocarditis prophylaxis	3	Penicillin V 250 mgm. t.d.s.	0.15 - 0.13 ug. 1 negative assay	Negative assay case relapsed
Cardiac surgery prophylaxis	2	Penicillin V 250 mgm. t.d.s.	0.3 - 0.04 ug.	No infections
Osteomyelitis follow-on	2	Penicillin V 250 mgm. t.d.s.	0.6 - 0.19 ug.	Remained well
Tonsillitis	12	Penicillin V 250-500 mgm. t.d.s.	1.2 - 0.05 ug.	7 cured with first course
Gonorrhoea	1	Penicillin V 250 mgm. t.d.s.	0.2 ug. at 3 hours	Cured

Table XI (continued).

Reason for Treatment	No. of cases	Penicillin and dose	Blood Level Range in Micrograms/ml. over 1-5 hours	Response
Osteomyelitis follow-on	2	Phenethicillin 250 mgm. t.d.s.	1.2 - 1 ug.	Remained well
Tonsillitis	10	Phenethicillin 250-500 mgm. t.d.s.	1.4 - 0.07 ug.	6 cured first course. 3 apparent cures relapsed
Acute bronchitis	9	Phenethicillin 250 mgm. t.d.s.	1 - 0.05 ug.	8 cured
Pneumonia	5	Phenethicillin 250 mgm. t.d.s.	2 - 0.3 ug.	4 cured
Acute sinusitis	1	Phenethicillin 250 mgm. t.d.s.	1 ug. at 2 hrs.	Cured
Otitis media	1	Phenethicillin 250 mgm. t.d.s.	0.1 ug. at 5-6 hours	Cured
Skin infections	4	Phenethicillin 250 mgm. t.d.s.	1.5 - 0.3 ug.	3 cured 1 abscess localisation

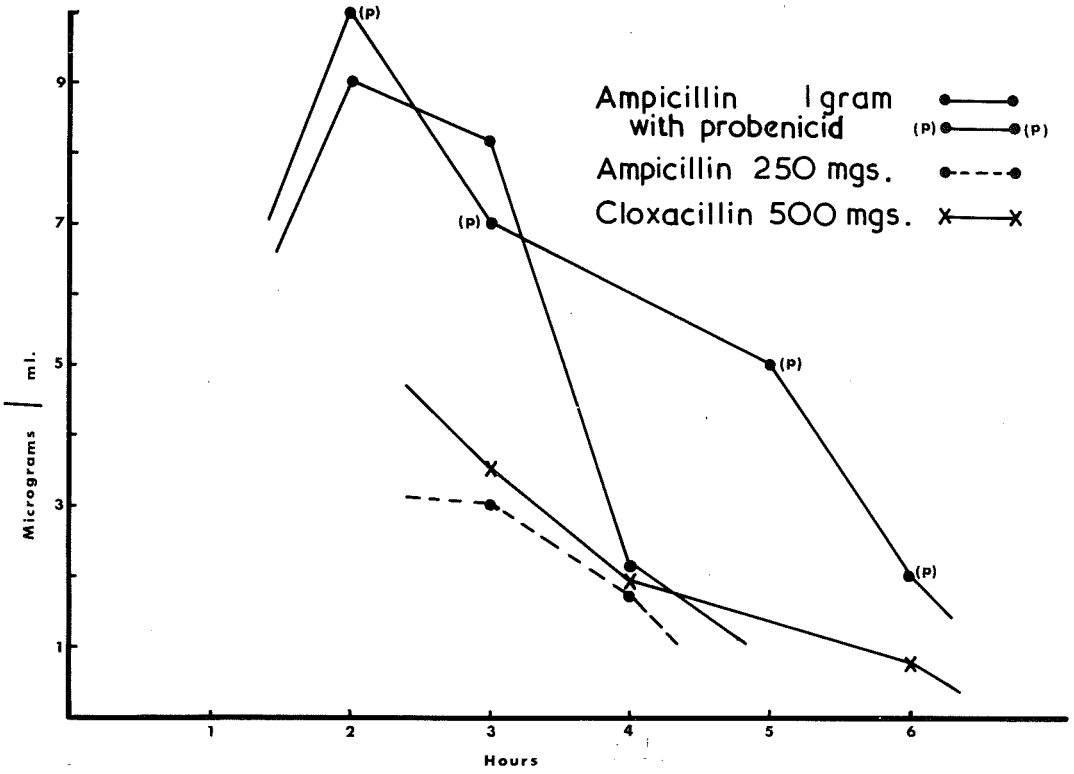


Figure 9. Mean blood levels of ampicillin and cloxacillin.

higher levels is apparent. Oral ampicillin curves have also been consistently more sustained than intramuscular injections in my experience. Ampicillin was therapeutically successful in the few acute bronchitis and urinary cases, but one typhoid carrier required cholecystectomy, and one Streptococcus faecalis endocarditis recovered slowly. Cloxacillin successfully cured the few penicillin resistant staphylococcal skin infections, but its usefulness as a parenteral drug could not be assessed as we find methicillin to be the safe and effective drug in serious conditions such as septicaemias.

CHAPTER IX.

CONCLUSIONS.

Laboratory Tests.

Strictly valid assays of penicillin in body fluids require many more controls than those used at present. They should include separate standard penicillins for blood and urine, both containing equivalent contents including metabolites, and they require strictly homologous diluents. A new overall control of pH would also be necessary when serum binding is taken into account, all specimens and diluents being tested and adjusted to physiological pH, or to the stable pH 8 of most *in vitro* sera. Another approach might be to separate penicillin from the test specimens, in a sephadex column, and then assay a total level in a stable buffer system.

In view of the difficulty of obtaining anything other than an approximate measure of expected therapeutic efficiency, the conclusion is reached that partly invalid assays, as performed at present, will be satisfactory for some time to come, provided that a constant technique is employed, utilising reliable standard penicillins in individual laboratories. Assays would include individual and comparative total blood levels and curves, and serum activity titres, and they should be correlated with a sound knowledge of pen-

icillin pharmacology and bacterial sensitivities. The assays should be expressed as weight of specific penicillin per unit volume, except perhaps for penicillin G, where the international standard makes it possible to use units of activity.

Free blood level estimations are shown in this thesis to be significantly higher than previous calculations or filtration measurements under inadequately controlled pH conditions. Routine tests for free levels on all specimens seem to be quite unnecessary, as easily performed total assays alone lead to a similar assessment of readily available penicillin from loosely bound blood reservoirs.

With regard to bacteriostatic sensitivity tests of the penicillins, my view is that all tests should be conducted in nutrient broth, with a minimum of added serum in the case of fastidious pathogenic organisms. Tube sensitivity tests performed with varying concentrations of serum are invalidated, with the exception of ampicillin, not by serum binding, but by the greatly increased penicillin destruction or inactivation. An 18 hour M.I.C. in serum is consequently of dubious significance.

Prolonged bactericidal tests from subcultures of tube titres, even in broth, also do not take into account the overnight incubation loss of antibiotic, and therefore are not valid. Six hour viable counts would seem to be the only reliable alternative.

Clinical Trials.

These have been criticised because of the lack of adequate controls, but the general picture has not changed over the years, and more recent double blind and cross-over trials of the newer penicillins have failed to produce much additional information.

Soluble penicillin G and all the new oral penicillins give reasonable results in clinical practice, particularly if common-sense is used in assessing which infections are suitable for oral therapy, and provided that adequate supervision of the oral regime is maintained. Under similar conditions, there should no longer be doubts as to the efficacy of intermittent therapy, indirect laboratory proof and satisfactory clinical responses being sufficiently convincing for most workers. True non-absorbers almost certainly do not exist, because repeat tests in these individuals invariably show some levels within accepted time limits, even if other irregularities such as late absorption are occasionally noted.

Future observation in clinical practice may influence the penicillins of choice, but the inherent pit-falls in oral therapy itself will probably always leave some factors at a clinical and statistical level.

Summary of Oral Penicillins and Proprietary Claims.

Penicillin G benzathine - tablets

capsules.

This preparation has been incorrectly claimed by one manufacturer to have a long-lasting effect, but this relates only to the prolonged intramuscular absorption of the insoluble salt. In the stomach this is rapidly hydrolysed to a soluble form, which only gives blood levels detectable over 4-5 hours with, at the most, another hour if taken after food.

This penicillin is not acceptable for oral therapeutic use, because the blood levels are consistently too low, in spite of the fact that mild streptococcal infections will usually respond. The only use recommended is 200,000^{u} b.i.d., before or after meals, for streptococcal prophylaxis in rheumatic fever subjects, where the same order of protection is obtained as with the sulphonamides and other oral penicillins. Some insist that a monthly injection of intramuscular benzathine G provides a better cover, but a suitable injection is not available on the Australian National Health Service (N.H.S.)

Penicillin G soluble

(crystalline benzyl penicillin) - tablets

capsules.

This product is cheap and, like oral benzathine G, obtainable in large quantities on the N.H.S. It is correctly

claimed to be as effective clinically as the phenoxypenicillins, if given in high dosage. Therapeutically, 400,000^{u} t.d.s. given before meals is acceptable for moderately established respiratory infections, and those of the skin if proven to be sensitive. The blood levels are definitely lower than with phenoxypenicillins, and the unbuffered tablets available here until recently would be more affected by careless usage with regard to meals and time of administration. These blood levels are again lower by a much greater factor than the potency of penicillin G is greater than the phenoxypenicillins. Also with the knowledge that most of the penicillins are easily and rapidly dissociated from their protein bound blood reservoirs, less penicillin G would be available from these lower levels for diffusion in such diseases as follicular tonsillitis, where high level penetration through inflammatory exudates is so often needed. Higher 800,000^{u} dosage could be used but the incidence of gastric intolerance and super-infections would be much higher.

Soluble penicillin G could also be prescribed for streptococcal prophylaxis, but tablets and capsules would not be quite as stable as the insoluble benzathine compound during prolonged periods of open storage.

Penicillin V potassium (phenoxymethyl penicillin)

calcium

benzathine

hydrabamine - tablets
capsules
powders
suspensions.

The manufacturers usually state that dependable levels are always found even in the presence of food. However, there are some irregularities in absorption particularly when taken after meals, but 250 mgm. t.d.s. for 7-10 days or 125 mgm. t.d.s. for children, can be recommended for moderate streptococcal and sensitive staphylococcal infections. Blood levels considerably higher than those obtained with penicillin G are attained, even in the presence of food, although strict supervision and at least one week of therapy is required. This supervision is even more necessary when penicillin V is used, with or without probenecid, in the follow-up of parenterally treated systemic infections, or in the full treatment of such infections in children.

Penicillin V is occasionally used for streptococcal prophylaxis, but being more expensive and in short supply for the N.H.S., it is not widely accepted.

Phenethicillin potassium
(phenoxyethyl penicillin) - tablets
capsules
suspension.

This product is stated to give higher tissue levels than penicillin V, from the evidence of higher total blood

assays and higher free levels measured by forced filtration. In this thesis, more accurate free levels can be calculated as being approximately equivalent, but the additional findings of rapid dissociation from the protein bound blood reservoirs confirms that most of the higher total levels of phenethicillin are almost certainly available for tissue diffusion. Slightly lower general biological potency and streptococcal sensitivities in particular are, however, found for phenethicillin, and it is finally difficult to recommend the drug to the exclusion of penicillin V.

It is claimed that some penicillinase-producing staphylococci will respond to phenethicillin owing to its slight resistance to the enzyme, and that "in practice patients do seem to get better quicker". In a situation where penicillinase-resistant penicillins would be available to private practice, these statements could not be supported, but where hospital restrictions to N.H.S. prescribing exist, their part-truth remains, whatever the wisdom of the present regulations.

Phenethicillin is usually prescribed in doses of 250 mgm. t.d.s. before or even after meals, and it has recently become similar in N.H.S. price to other penicillins. Some have the impression that in adults capsules cause less dyspepsia than tablets, because they obviate some of the occasional need of prescribing antacids. A point in favour of

phenethicillin is the fact that no negative blood assays have been recorded in my clinical assays nor in published figures to my knowledge, whereas both sources have a small percentage of negative results for penicillin V, due no doubt to the levels being below measurable limits.

Claims of equality to equivalent intramuscular doses of soluble penicillin G seem superfluous, as one can rely upon more regular absorption from the parenteral product, which in any case should be given in the early period of any established infection causing doubts concerning the wisdom of using oral therapy (e.g. otitis media).

Ampicillin acid - tablets

capsules.

This antibiotic is claimed to be a broad-spectrum penicillin, and it is effective in chest infections due to Gram positive cocci and Haemophilus influenzae, acute urinary tract infections due to sensitive Gram negative bacilli, and acute systemic infections with Streptococcus faecalis, Salmonella typhi and Haemophilus influenzae. It produces rapid and high blood levels, but the treatment of salmonellosis has been disappointing.

Doses vary from 250 mgm. - 1 gram t.d.s. or 6-hourly, and the drug is obtainable under hospital restrictions of the N.H.S., at a high cost to the Government. New cephalosporin derivatives with a similar spectrum of activity are as yet unsuitable for oral use.

Cloxacillin sodium

(similar to oxacillin) - capsules

syrup.

This drug is highly bactericidal to both sensitive and resistant staphylococci, and it is used with reasonable effect in doses of 500 mgm. t.d.s. preferably before meals. Fucidin is somewhat cheaper and equally effective, but *in vivo* resistance is feared, and both drugs have to be carefully assessed against the clinical situation before they are used to the exclusion of parenteral products.

Propicillin and Phenbenicillin

(phenoxypropyl and phenoxybenzyl penicillins).

These antibiotics have no advantage over penicillin V and phenethicillin and they are not available in Australia. Phenbenicillin also shows some indirect evidence of stronger protein binding, with the result that total levels would probably not be available for tissue diffusion.

SUMMARY.

In view of the multiplicity of oral penicillins and the lack of adequate knowledge concerning specific products and their indications, a broad study of the oral literature, laboratory and clinical practice has been undertaken.

This study gathers the oral penicillins into one organised subject, and it is original as such. It summarises the pharmacology and pharmaceutics pertaining to oral administration, and the results of many serum assays conducted and assessed by myself and others from a wide range of clinical cases.

A fresh investigation by me and Dr. J. R. Coulter of the serum binding of penicillin has produced some original contribution to the understanding of binding effects in the laboratory and *in vivo*. The pH of test fluids used is quite critical, and by strictly controlled tests at physiological pH we have shown binding percentages much lower than those of previously published work. The penicillins have also proved to be loosely bound to serum protein and a large portion of total blood levels are thought to be available for tissue diffusion.

Soluble penicillin G, penicillin V and phenethicillin, give reasonable results as oral antibiotics in clinical practice when assessed by me and others, although penicillin G shows persistently lower blood levels, particularly with

careless oral usage in regard to meals, time and length of administration. Oral penicillin should be prescribed only for moderate streptococcal and sensitive staphylococcal infections, and parenteral drugs should be used at least in the early period of fully established infections. Oral ampicillin is effective in the general range of acute urinary tract and chest infections, and cloxacillin is useful in moderate penicillin-resistant staphylococcal sepsis.

The acceptance of oral medication as a reliable therapy has been resisted by some physicians. However, doubts concerning the efficacy of intermittent therapy are no longer warranted in the presence of adequate supervision, because indirect laboratory evidence sufficiently supports the validity of discontinuous oral treatment, and clinical responses are satisfactory. Concern about the irregular though reliable absorption of oral products is also unnecessary as truly non-absorbing individuals probably do not exist, repeat tests after negative assays invariably showing measurable blood levels.

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APPENDIX I.

Proprietary Oral Penicillins available in Australia.

Penicillin G benzathine - Bicillin tablets
L.P.G. tablets
Benzathine penicillin capsules

Penicillin G soluble (crystalline) - Crystapen tablets
Abbocillin G
capsules
tablets (buffered)
Penevan capsules

Penicillin V potassium - Penicillin V tablets
capsules
P.V.O. tablets
Peni-Vee K tablets
Cilicaine VK tablets
capsules
Distaquaine VK tablets
PVK tablets
pulvules
pedipacs
oral suspension
Propen VK tablets
CVK tablets
capsules
Falcopen VK tablets
capsules

Abbocillin VK film tablets

capsules

Distakaps VK capsules

Tab-Pen VK

Veecillin tablets

CVL oral suspension

Citrapen suspension

Penicillin V calcium - Penicillin V tablets

Caps Pen V

Sus - Pen V

Peni-Vee suspension

Veecillin oral suspension

Penicillin V benzathine - Vicin tablets

sachets

aqueous suspension

Cilicaine V oral suspension

Bicillin V aqueous suspension

Falcopen V aqueous suspension

L.P.V. suspension

Penicillin V hydrabamine - Abbocillin V aqueous suspension

Phenethicillin - Pensig tablets

capsules

oral suspension

Optipen tablets

capsules

Ampicillin - Penbritin capsules

tablets

syrup

Austrapen capsules

Cloxacillin - Orbenin capsules

syrup

Austrastaph capsules.

APPENDIX II.

ORIGINAL WORK INDEX.

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ORAL PENICILLINS IN CLINICAL PRACTICE

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ORAL PENICILLINS IN CLINICAL PRACTICE

A. W. DERRINGTON, M.B., B.S., D.T.M. and H.[1]

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THE multiplicity of oral penicillins has resulted in some confusion among medical practitioners as to the relative merits of specific products. As each new semi-synthetic penicillin has appeared, many physicians have depended on the limited knowledge of the drug detailers, and ineffective oral prescribing has only too frequently been the consequence.

A perusal of the literature reveals the persistence of doubts concerning non-absorbing patients, continuing fears in regard to intermittent dosing, and difficulties in correlating in-vitro tests with in-vivo situations. Repeated laboratory studies in medical volunteers and variously controlled clinical trials have shown the phenoxypenicillins to be effective in properly selected infections, but with no clear-cut preference over soluble benzylpenicillin (penicillin G). The recent aminobenzyl penicillin (ampicillin) has already proved its usefulness in the broad-spectrum field, but the isoxazolyl oral penicillin (cloxacillin) appears to be only moderately effective against resistant staphylococcal infections.

In view of this unresolved situation, a broad re-study of the principles and practice of oral medication, including penicillin pharmacology, has been attempted and presented as an M.D. thesis. This article deals briefly with the pharmaceutical claims of available Australian products, and gives a clinical assessment of many serum assays of different penicillins prescribed for patients in private and hospital practice. Laboratory studies, including a fresh examination of serum binding and its in-vivo significance, will be reported elsewhere (Coulter and Derrington, 1965).

MATERIALS AND METHODS

Representative oral penicillins available in Australia were briefly tested in regard to biological potency and acceptability of tablet disintegration. Penicillin assay standards were supplied as subsidiary standards from the Commonwealth Serum Laboratories, and they consisted of "Penicillin G (Potassium)", "Penicillin V (Acid)" and phenethicillin potassium. Beecham's Research Laboratories provided working standards of ampicillin ("Penbritin") and cloxacillin ("Orbenin"). Sera and blood were received one to five hours after the last preprandial dose, from general hospital and private practice, many patients having several assays performed on different occasions. The sera were separated on the same day and stored at -20° C.

A *Sarcina lutea* plate diffusion assay was adapted from Grove and Randall (1955) for the estimation of total penicillin serum levels, and then modified for frequent serum samples collected over many months. Duplicated agar cup tests were conducted in batches, against a standard curve of specific penicillin diluted in the serum substitute 2% albumin (fraction V). Standard curves were renewed every one or two weeks with each new lot of assay medium or seed stock. Assay limits of error were worked out from six randomized duplications for each of the three standard curve points, and these amounted to ±18%. This was an acceptable limit for clinical assays as applied to in-vivo situations impossible of really accurate definition. The amount of benzylpenicillin has been expressed in units per millilitre because there is an international standard of known biological activity (1 international unit being equal to the activity contained in 0·6 μg.). As the new penicillin derivatives cannot validly be assayed against benzylpenicillin, they were assayed against the specific penicillin and expressed in micrograms per millilitre.

The Grove and Randall *Staphylococcus aureus* potency test was used, and a tablet disintegration test was adapted from the British Pharmacopœia test-tube inversion method. Personal sensitivity tests were performed by the serial tube dilution method, again of Grove and Randall.

RESULTS

All products tested were fully potent, and the tablets proved to be soft or only moderately hard. Percentage potencies usually varied between 95 and 105 for both tablets and capsules, while the tablets disintegrated in 2 to 15 minutes in water and in 0·1% hydrochloric acid. Approximately half the harder "Benzathine G" tablets (Wyeth) and the more resistant film tablets (Abbott) remained after 20 minutes' shaking in 0·1% hydrochloric acid, although this did not appear to lower absorption rates.

Soluble or crystalline benzyl penicillin specimens were assessed in low 200,000-unit dosage from some volunteer trials and a few private prescriptions for bronchitis, with higher 400,000-unit dosages coming from cases of tonsillitis. Insoluble "Penicillin G (Benzathine)", 200,000 units, was assayed from patients under streptococcal prophylaxis

[1] Medical Bacteriologist.

for rheumatic fever in private practice. Figure I confirms that "Benzathine G" levels were lower than the equivalents found for soluble "Penicillin G", and no indication was found of the prolonged effects sometimes claimed for the former. There were a few assays with the insoluble salt that produced negative results, but on further tests these subjects proved to be positive absorbers either with the same product or with "Penicillin V".

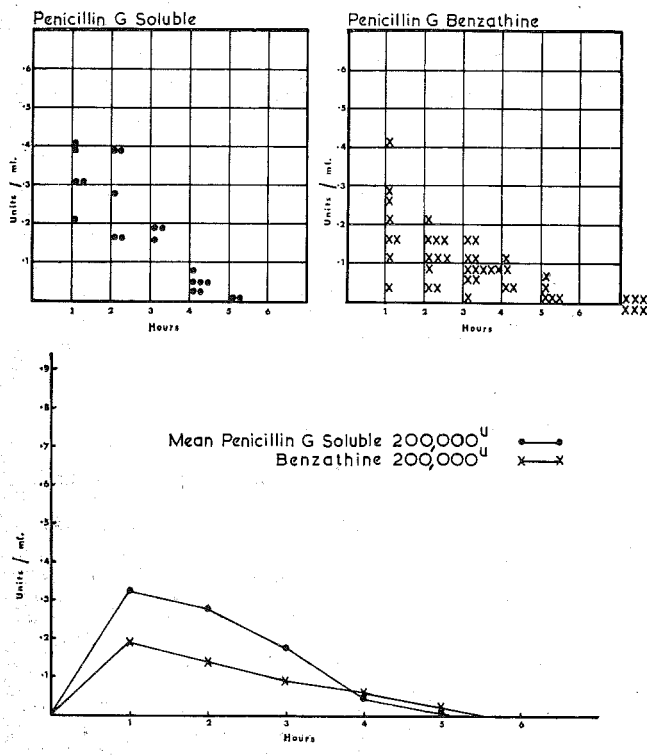

FIGURE I: Individual and mean serum assays of oral "Penicillin G".

"Penicillin V (Potassium)" and phenethicillin assays were from hospital patients on 250 mg. doses. Figure II shows that phenethicillin is better and more regularly absorbed than "Penicillin V", and unlike the latter, it did not produce any negative assay results under the conditions of this trial. Blood levels were generally a little lower than those of previous volunteer trial publications, but they were still well above equivalent levels of "Penicillin G (Soluble)".

Common dosages of the oral penicillins are compared in Figure III as average blood levels from some previous studies. A comparison of average minimum inhibitory concentrations for the usual pathogens is also listed in Table I. Figure IV shows graphically the average plot of a small number of ampicillin and cloxacillin assays, very good blood levels being noted for ampicillin. Probenecid did not greatly raise early levels of orally administered ampicillin under these conditions, but some prolongation of high levels is apparent. Probenecid in fact approximately doubles and prolongs the blood levels of all the penicillins only if it is given with the frequency and regularity necessary to overcome the penicillins' extremely rapid kidney excretion.

Clinical responses from a wide range of assay specimens are presented in Table II. Both "Penicillin G (Soluble)" and "Penicillin G (Insoluble)" are seen to be acceptable for streptococcal prophylaxis, and they are much cheaper than the phenoxypenicillins. The therapeutic value of "Penicillin G (Soluble)", ampicillin and cloxacillin is not assessable from this investigation, but "Penicillin V" and phenethicillin show their usefulness in moderate streptococcal infections and in penicillin-sensitive staphylococcal infections.

DISCUSSION AND CONCLUSIONS

This report confirms the general picture obtained from a study of oral penicillin history. "Penicillin G (Soluble)"

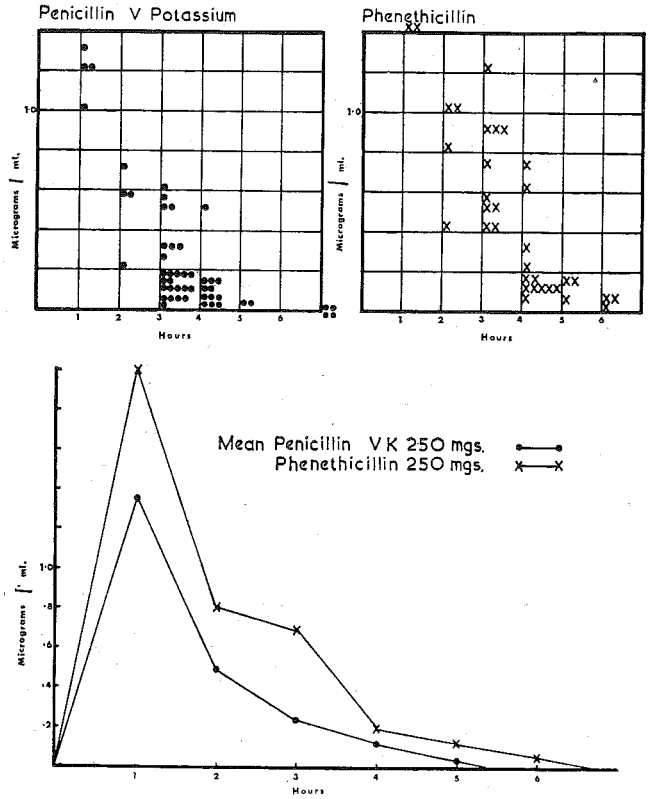

FIGURE II: Individual and mean serum assays of oral phenoxypenicillins.

and the new penicillins give reasonable responses in clinical practice for moderately established infections, particularly if oral prescribing is well supervised.

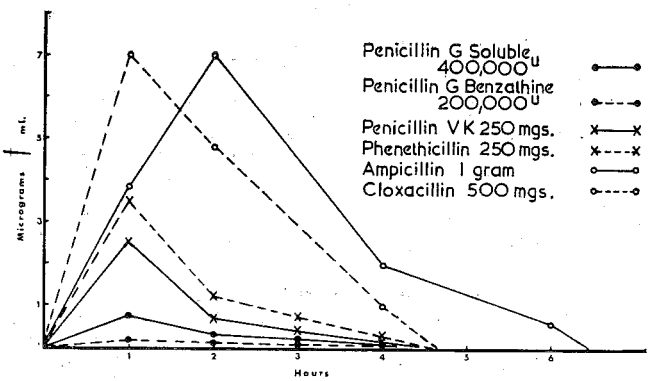

FIGURE III: Average serum assays from previous publications; oral dosages in common use. (Jones and Finland, 1955; Putman *et alii*, 1955; Peck and Griffith, 1955; Juncher and Raaschou, 1957; Morigi *et alii*, 1959; Cronk *et alii*, 1959; Knudsen and Rolinson, 1959; McCarthy and Finland, 1960; Knudsen *et alii*, 1961; Knudsen *et alii*, 1962.)

Continuing doubts concerning discontinuous therapy are needless; indirect laboratory evidence and good clinical recoveries are sufficient proof of the effectiveness of intermittent dosing. The few non-absorbers are almost certainly more apparent than real, the explanations being found in

irregular absorption or dosing particularly in regard to time and meals, late sampling, and variable late absorption in patients with pyloric delay. Normal total blood levels are assayable for three to five hours after an oral dose, and when these levels are four or more times above the minimum inhibitory concentration in broth of specifically tested or known pathogen sensitivities, the in-vivo assessment is generally correct. The few response failures are mostly revealed as difficulties in effective oral supervision or tissue penetration of the antibiotics.

TABLE I

Average Broth Minimum Inhibitory Concentrations (Microgrammes per Millilitre)[1]

Infecting Organism	"Penicillin G"	"Penicillin V"	Phenethicillin	Ampicillin	Cloxacillin
Staph. aureus (sensitive) ..	0·02	0·03	0·03	0·05	0·15
Staph. aureus (resistant) ..	—	—	—	—	0·4
Strep. pyogenes ..	<0·02	0·02	0·03	0·02	0·07
Strep. pneumoniæ ..	0·02	0·02	0·06	0·02	0·25
Neisseria gonorrhœæ	0·02	0·03	0·12	0·13	0·5
N. meningitidis ..	0·03	0·12	—	0·06	0·5
Clostridium welchii	0·06	—	—	—	—
Strep. fæcalis ..	2	4	4	2	25
H. influenzæ ..	1	4	4	0·25	16
Salmonella species ..	8	128	>250	2	—
Escherichia coli ..	64	128	>250	6	—
Proteus mirabilis ..	—	—	—	4	—
P. vulgaris	—	—	—	64	—

[1] Gourevitch *et alii*, 1959; Rolinson and Stevens, 1961; Barber and Waterworth, 1962; Knudsen *et alii*, 1962; Bond *et alii*, 1963; and personal tests.

"Penicillin G (Benzathine)": Tablets and Capsules

This antibiotic is incorrectly stated by one manufacturer to have a long-lasting effect when given by mouth. This refers only to intramuscular release of the insoluble salt, because the oral form is rapidly hydrolysed to the soluble form in the stomach. This penicillin is not acceptable for oral therapeutic use, because blood levels are consistently too low. In spite of the fact that mild streptococcal infections will often respond, it should be used only in doses of 200,000 units twice a day for the streptococcal prophylaxis of rheumatic fever, in which the same order of adequate protection is obtained as with sulphonamides and other oral penicillins (Alexson *et alii*, 1960).

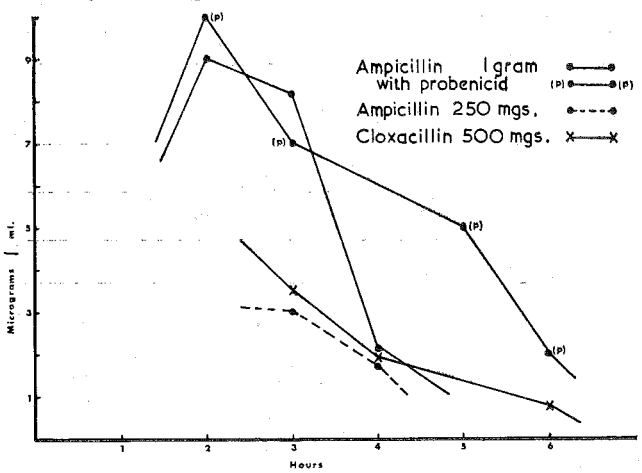

FIGURE IV: Clinical assays of ampicillin and cloxacillin.

"Penicillin G (Soluble)", Crystalline: Tablets and Capsules

This product is cheap, and like "Penicillin G (Benzathine)" for oral use, it is obtainable in large quantities on the Australian National Health Service. Therapeutically, a dosage of 400,000 units (250 mg.) three times a day before meals is prescribed in such conditions as moderately established respiratory tract infections, and in skin infections proved to be sensitive to penicillin. This antibiotic is relatively acid-labile, and the blood levels obtained, especially with the unbuffered tablets generally available here, are not only lower than those of the phenoxypenicillins, but are more affected by the inherent trend of oral therapy to careless usage in regard to meals and the consequent higher stomach acidity (Jones and Finland, 1955).

"Penicillin G (Soluble)" is probably not as effective as the phenoxypenicillins in acute fully established tonsillitis, as the lower blood levels would not provide such a high-level penetration of excess inflammatory products. High dosages (800,000 units) could be prescribed, but there would be more difficulty with gastric intolerance and throat and bowel superinfections. However, this product can be recommended for prophylaxis against the streptococcus in doses of 200,000 units twice a day.

"Penicillin V (Potassium)" or "Penicillin V (Calcium)" and "Benzathine" or "Hydrabamine": Tablets, Capsules, Powders and Oral Suspensions

The manufacturers often quote the dependability of the absorption of acid-stable phenoxymethyl penicillin in the presence of food. There are, however, irregularities in absorption of "Penicillin V", and especially so after food (Juncher and Raaschou, 1957). The recommended dose is 250 mg. three times a day for seven to 10 days, or 125 mg. for children, in suitable streptococcal and sensitive staphylococcal infections. Higher blood levels than those of "Penicillin G" are attained, even in the presence of food; but strict supervision of oral therapy is necessary, particularly when one may wish to manage serious infection in a child with oral medication.

"Penicillin V" is occasionally prescribed for prophylaxis against streptococci, but since it is much dearer and shorter in supply on the N.H.S. than "Penicillin G", its use should not be considered.

Phenethicillin Potassium: Tablets, Capsules and Oral Suspension

Phenoxyethyl penicillin is stated to produce higher tissue levels than "Penicillin V", because Bond *et alii* (1963) found higher free serum levels in a comparative study. The total serum levels of phenethicillin are in any case greater, and loose serum binding would seem to permit a large portion of the overall total to diffuse into the tissues. In the final analysis, the slightly lower streptococcal sensitivities to phenethicillin make it difficult to recommend one drug over another.

Some of the penicillinase-producing staphylococci will respond to phenethicillin's slight resistance to the enzyme (Gourevitch *et alii*, 1959; Edgar, 1960), and in private practice, when the penicillinase-resistant penicillins are not available on the N.H.S., some skin infections and respiratory tract complications may generally respond better to this drug. Another point in favour of phenethicillin is the fact that no negative results to serum assays have been recorded in the clinical assays concerned in this report, and none from published figures to my knowledge. However, "Penicillin V" has shown a small percentage of such findings from both sources, this being no doubt due to less regular absorption and unmeasurable low levels.

Phenethicillin is usually prescribed in a dosage of 250 mg. three times a day, preferably before meals, but it is a little more expensive for the N.H.S. Claims of equality to equivalent intramuscular doses of "Penicillin G (Soluble)" seem superfluous, as one can always rely on the more certain and regular absorption of parenterally administered products. "Penicillin G (Soluble)" should be injected in any case in the early treatment of fully-established infections such as tonsillitis and streptococcal

TABLE II
Clinical Responses to Oral Penicillins

Reason for Treatment	Number of Cases	Penicillin	Blood Levels	Response
Rheumatic fever prophylaxis	18	"Benzathine G", 200,000 units twice a day	4 Negative results; 1 very low, changed to "Penicillin V".	No recorded relapses
Rheumatic fever prophylaxis	10	"Penicillin V", 250 mg. twice a day	2 Negative results	1 Relapse from irregular medication
Streptococcal throat prophylaxis for other complications	3	"Benzathine G", 200,000 units twice a day	Acceptable levels	All relapsed when tablets left off
Cardiac surgery prophylaxis	2	"Penicillin V", 250 mg. three times a day	Good levels	No infections
Subacute bacterial endocarditis prophylaxis	2	"Penicillin V", 250 mg. three times a day	1 Negative result	1 Relapse
Subacute bacterial endocarditis prophylaxis	1	Phenethicillin, 250 mg. three times a day	Good level	Remained well
Subacute bacterial endocarditis treatment	1	Ampicillin parenterally followed by 1 gramme six-hourly orally with probenecid	Variable levels	Slow recovery from *Strep. fæcalis*
Streptococcal throat infection and tonsillitis	3	Soluble "Penicillin G", 400,000 units three times a day	Acceptable levels	1 Superinfection with *E. coli*
Streptococcal throat infection and tonsillitis	12	"Penicillin V", 250–500 mg. three times a day	Good levels	7 Cured
Streptococcal throat infection and tonsillitis	10	Phenethicillin, 250–500 mg. three times a day	Good levels	6 Cured; 3 apparent cures, relapse
Acute bronchitis	9	Phenethicillin, 250 mg. three times a day	Good levels	8 Cured
Acute bronchitis	2	Ampicillin, 250 mg. three times a day	Good levels	2 Cured
Chronic bronchitis prophylaxis	5	Soluble or "Benzathine G", 200,000 to 400,000 units twice a day	Good levels	1 Repeatedly relapsed on "Benzathine"
Pneumonia	5	Phenethicillin, 250 mg. three times a day	Good levels	4 Cured
Acute sinusitis	1	Phenethicillin, 250 mg. three times a day	Good levels	Cured
Otitis media	1	Phenethicillin, 250 mg. three times a day	Good levels	Cured
Skin infection	4	Phenethicillin, 250 mg. three times a day	Good levels	4 Cured
Skin infection	2	Cloxacillin, 500 mg. three times a day	Good levels	2 Cured
Urinary infection	3	Ampicillin, 250 mg. three times a day	Good levels	3 Cured
Gonorrhœa	1	"Penicillin V", 250 mg. three times a day	Good levels	Cured
Typhoid carrier	1	Ampicillin, 1 gramme six-hourly	Good levels	Required cholecystectomy
Osteomyelitis—follow-on treatment	2	"Penicillin V", 250 mg. three times a day	Good levels	Remained well
Osteomyelitis—follow-on treatment	1	Phenethicillin, 250 mg. three times a day	Good levels	Remained well
Osteomyelitis—follow-on treatment	3	Cloxacillin, 500 mg. three times a day	Good levels	Remained well

otitis media. "Procaine Penicillin G" injections, with lower but more prolonged serum levels, also have some use in these situations before follow-up therapy is undertaken with an orally administered penicillin.

Ampicillin Acid: Tablets and Capsules

This is stated to be a broad-spectrum penicillin, and the relatively high blood levels it achieves are effective in chest conditions, and in acute urinary tract infections from sensitive Gram-negative bacilli (May, 1964; Stratford, 1964). Systemic infections with *Strep. fæcalis*, *Salmonella typhi* and *Salmonella paratyphi*, and *Hæmophilus influenzæ* are also responsive to the drug.

Oral doses vary from 250 mg. three times a day for urinary infections to 1 gramme three times a day or every six hours for the systemic infections, but unfortunately the drug is expensive and obtainable only under hospital restrictions of the N.H.S. New cephalosporin derivatives are of a similar spectrum, but they are as yet unsuitable for oral absorption.

Cloxacillin Sodium: Capsules and Syrup

This drug, similar to oxacillin, is highly bactericidal to both sensitive and resistant staphylococci, and it is used with reasonable effect in doses of 500 mg. three times a day before meals (Stratford, 1963). However, fully established or systemic infections should be treated by the parenteral product or preferably methicillin (May *et alii*, 1964). Another effective oral antibiotic in this category is fucidin, but the development of in-vivo tolerance is feared, and like cloxacillin it is an N.H.S. hospital restriction.

Propicillin and Phenbenicillin

These later phenoxypenicillins appear to hold no advantages over the other penicillins, and they are not available on the N.H.S. or in Australia.

SUMMARY

1. The oral penicillins available in Australia are examined as a group.

2. Clinical assays and therapeutic responses are presented.

3. Clinical indications and usage are discussed.

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