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Review Article

Pharmaceutics of Penicillin

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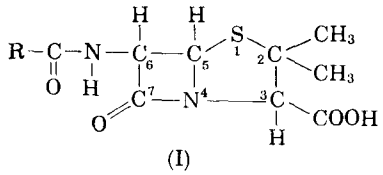
PROBABLY no group of drugs has received more attention and been more intensively studied than the penicillins (I).



(I)

Their basic structure includes a double ring thiazolidine β-lactam moiety which has been named 6-aminopenicillanic acid (6-APA). The differences in the physical, chemical, and biological properties of the various penicillins are due to their side chains (R in I).

The β-lactam ring is quite labile in all the penicillins known at present. Differences in stability of the several penicillins are determined by the side chains, the structural characteristics of which confer varying degrees of protection against degradation.

Before the isolation of 6-APA from fermentation broths by a group at the Beecham Laboratories in England in 1958 (1), complete penicillins could be made commercially only by fermentation, and variations in the side chain were made by the addition of appropriate precursors to the fermentation broth. This severely limited the number and types of penicillins which could be produced. Since 1958, however, penicillins have achieved a revived importance in chemotherapy because now, theoretically any side chain may be linked by chemical means with the 6-APA nucleus to form a new penicillin. In addition, other workers (2–4*a*) have found enzymes which will selectively hydrolyze penicillin at the amide linkage to produce 6-APA.

Because of this revived interest in penicillins, their stability and incompatibilities with other medicinals have become of major importance to pharmacists in every area of the profession. It is the purpose of this paper to review and correlate all the available data on penicillin stability. Penicillin G (I, R = $C_6H_5CH_2$—), on which most of the work has been done, will be discussed first, and then the newer penicillins compared with it.

PENICILLIN G

Routes of Degradation.—Figure 1 depicts the major routes of degradation of penicillin G of importance in pharmaceuticals. In neutral or alkaline medium it is hydrolyzed at the β-lactam to penicilloic acid (II) (5). The reaction at constant temperature and pH is first order with respect to penicillin and is directly proportional to hydroxyl ion concentration (6). Benzylpenicillin is much more reactive toward alkali than simpler β-lactams, and this has been attributed to facilitation of hydrolysis by the sulfur bonded to the β-lactam ring and/or fusion of the β-lactam ring with a five-membered ring (7). The mechanism of this reaction in neutral solution is not completely understood, but several workers (8, 9) have obtained evidence from ultraviolet absorption data that it proceeds through penicillenic acid (III). This compound has been isolated and shown to have a maximum

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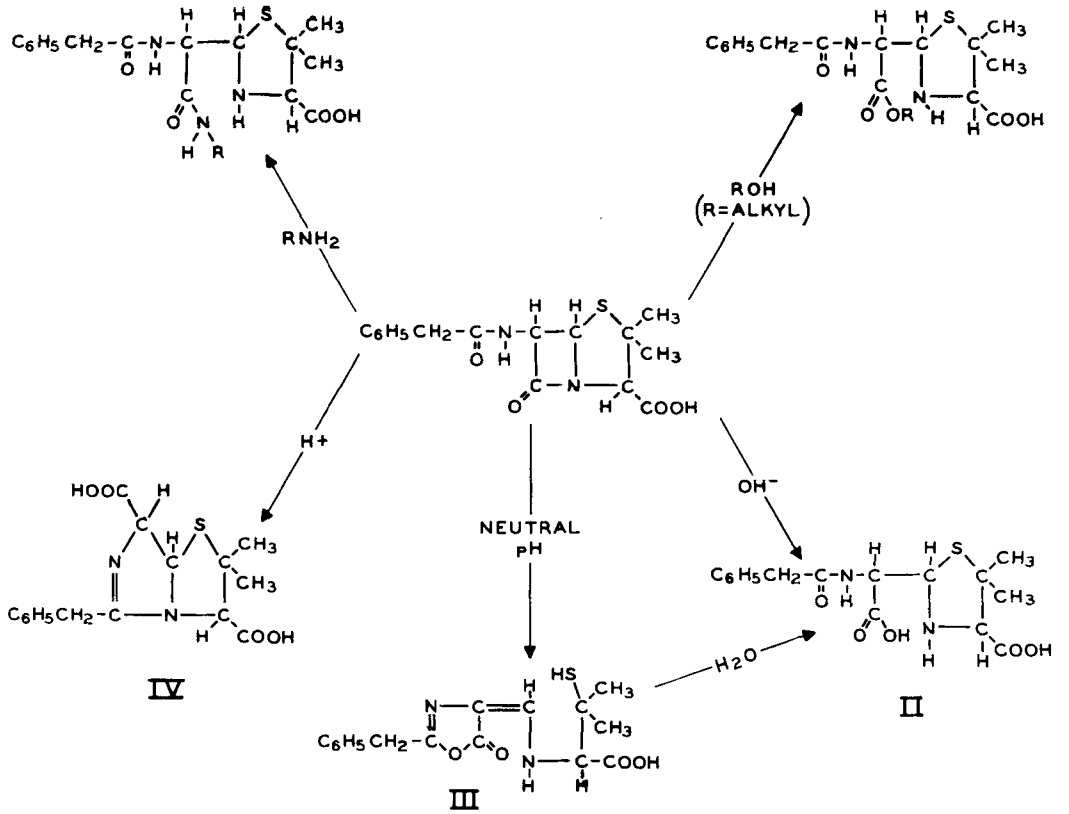


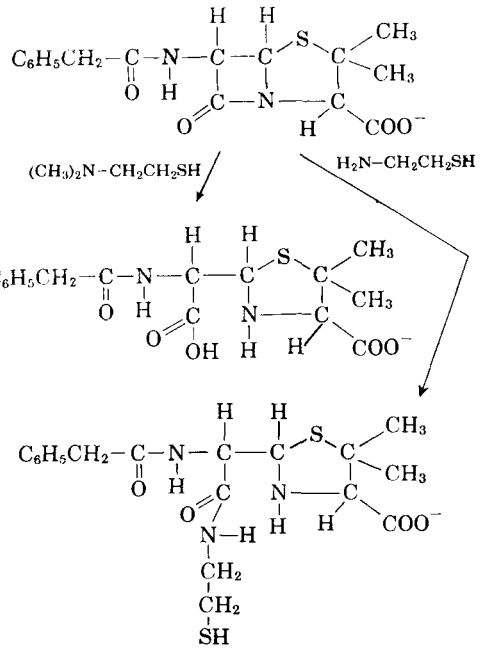
Fig. 1.—Major degradative reactions of penicillin.

in its ultraviolet absorption spectrum at 322 mμ (10). During hydrolysis of penicillin at pH 7.5 the absorption at 322 mμ increases, indicating the presence of this compound. It is rapidly converted to penicilloic acid with half-life, at pH 7.5 and 37°, of 6.5 minutes (10). The overall half-life of penicillin G under these conditions is about 60 hours. In strongly alkaline solution, penicillin G is probably hydrolyzed directly to penicilloic acid. In strong acid solution, penicillin is rearranged to penillic acid (IV) (5). At constant temperature and pH, this reaction is also first order (11) and depends on hydrogen ion concentration (12–14). This dependence is not linear due to the ionization of penicillin which has a pKa about 2.8 and, therefore, exists in two forms, free acid and ion, in the pH range from about 1.5 to 4.5. Each of these species is inactivated at a different rate, and this produces the curvature in the pH profile of Fig. 2. Similar pH dependence, although at different rates, has recently been demonstrated for phenethicillin (15) (I, R = C_6H_5OCH(CH$_3$)—) and this is also shown. Penicillins F, K, and X, all of which were made by fermentation, are also inactivated at different rates (16), but are not important therapeutically.

From the pH profile it may be observed that the pH of minimum degradation is about 6.5. This naturally is quite important in formulation of pharmaceuticals.

Penicillin reacts fairly rapidly with certain alcohols and amines to form the esters and amides, respectively, of penicilloic acid (5).

The reaction of penicillin with cysteine and some related mercaptoamines has been investigated from a mechanistic standpoint (17). With primary mercaptoamines, the products were found to be N-penicilloylamides, whereas with tertiary mercaptoamines the product was free penicilloic acid. From the pH dependence of the reaction it was concluded that the active species was a mercaptide ion and the rate-limiting step was formation of a semimercaptol intermediate. In the case of the primary mercaptoamines, this intermediate rearranged to the amide. With the tertiary mercaptoamine, amide formation was impossible and the product was penicilloic acid.



Some other reactions of penicillin of pharmaceutical interest will be discussed below in the section on imcompatibilities.

One other route of degradation which is more important therapeutically, but which deserves mention here, is the hydrolysis of penicillin by penicillinase. This enzyme is formed by certain microorganisms, notably certain strains of staphylococci, and rapidly catalyzes the hydrolysis of penicillin to penicilloic acid. The reaction is known to follow the usual Michaelis–Menten kinetics (18, 19). Certain compounds have been shown to inhibit penicillinase *in vitro* (20–24) but none of these has been used therapeutically.

STABILITY OF PENICILLIN IN DOSAGE FORMS

Solid Dosage Forms.—The most critical factor governing stability of penicillin in the solid state is moisture content. In one study of crystalline sodium penicillin G (25) it was shown that when the moisture content in vials was above 4%, significant loss of potency (40–80%) occurred after 6 months at 25°. This effect was much less pronounced at refrigerator temperature. Thus, the hygroscopic capacity of the powder and the type of closure on the vial are extremely important (26).

In the early days of penicillin amorphous forms of the salt were often used. It was generally found that the crystalline forms were much more stable than the amorphous, although this could not be correlated with moisture content (27). It was also found that the calcium salt was less hygroscopic than the sodium salt (28). Nevertheless, calcium penicillin has not seen wide usage.

A study of many commercial lots of crystalline sodium and potassium penicillin in vials showed that there were only slight losses (1.5–2%) after 3 years storage at room temperature (29). Thermolability of sodium penicillin has been followed by several workers (30, 31) and it was found that the antibiotic could be heated at 100° for 4 days with no more than 10% loss in activity. At temperatures over 140° however, loss was quite rapid.

Relatively insoluble amine salts of penicillin, such as the procaine and dibenzylethylenediamine salts, are also stable as dry powders for periods of 3 years or more at room temperature (32), as are combinations of procaine and potassium

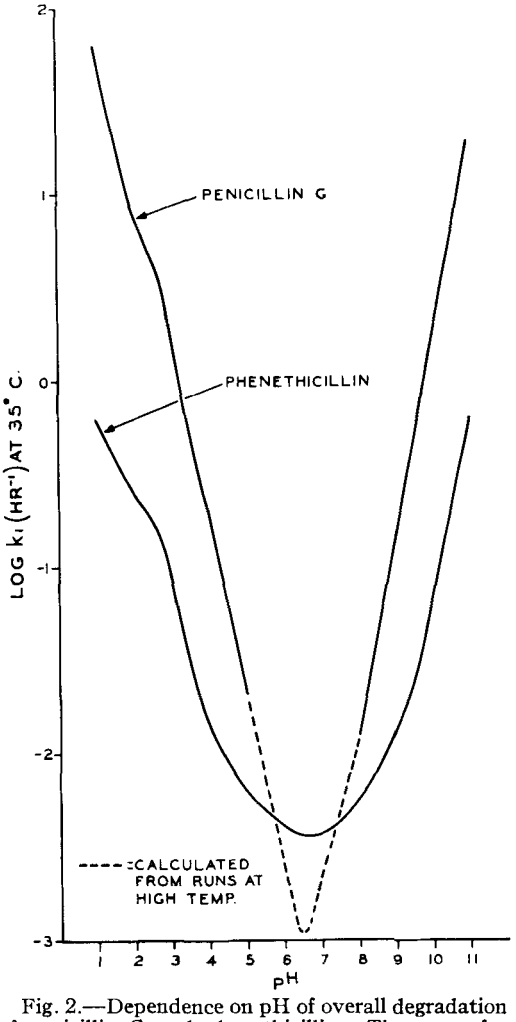


Fig. 2.—Dependence on pH of overall degradation of penicillin G and phenethicillin. The curve for penicillin G was taken from ref. 16.

penicillins (33). Buffered penicillin powder, marketed for oral use after reconstitution with water, and combined with flavors, dyes, and sweeteners, was found to exhibit virtually no loss in potency after storage at room temperature for 4 years (34).

The stability of penicillin tablets is also dependent on moisture content. An early report (35) showed that tablets packaged with silica gel were much more stable than those packaged without the moisture absorber. Later it was shown that buffered penicillin tablets (36) and soluble tablets in hermetically sealed aluminum foil (37) were stable for at least 3 years, as were troches of potassium and procaine penicillin (38, 39). It has also been stated that sugar coating improves the stability of penicillin in tablets (40).

Liquid Dosage Forms.—As noted in referring to the kinetic studies of the degradation of penicillin in aqueous solution, the antibiotic is destroyed fairly rapidly and this reaction is catalyzed by both acid and base. It has been noted also that the reaction in neutral solution produces acid (penicilloic). From a glance at the titration curve of penicillin in Fig. 3 it can be seen that a small amount of acid will lower pH very rapidly. Thus, in a solution of penicillin in plain water, one may expect the rate of hydrolysis to increase as the initial reaction lowers pH to a point where the acid-catalyzed reaction becomes appreciable. It was recognized quite early that it was necessary to buffer solutions of penicillin near neutral pH in order to obtain optimum stability (41, 42). A controversy arose as to whether phosphate or citrate was the better buffer system. Hahn (43) claimed that citrate stabilized penicillin to a greater extent than would be indicated by its buffering action alone. He compared stability in citrate and phosphate buffer of the same pH and found that the solution in citrate buffer was more stable. Pulvertaft and Yudkin (44) claimed that phosphate also stabilized penicillin beyond its buffer effect yet, at the same time, showed that there existed an optimum concentration of phosphate for a particular concentration of penicillin. If phosphate were exerting some stabilizing effect, one would expect higher concentrations to stabilize even more so. The fact that higher concentrations caused less stability suggests that phosphate buffer had some catalytic effect on hydrolysis of penicillin. Pratt (45) found that phosphate buffers of a particular concentration stabilized penicillin better than lower concentrations, but found no difference in half-life when concentrations above his "optimum" were used. This indicates that the lower concentrations were simply of too low a buffer capacity for the concentration of penicillin used. Thomas (46) claimed that phosphate, citrate, and acetate buffers all stabilized penicillin. On the other hand, Macek, *et al.* (47), presented evidence which showed that the stabilizing effect of phosphates and citrates depends on their buffering action and not on any specific ion effect. Other workers (48, 49) have shown that increasing concentrations of penicillin require increasing concentrations of phosphates to maintain optimum stability. It thus appears that the action of buffers in stabilizing penicillin is strictly related to their ability to maintain pH. It should be noted here that the penicillin available at the time most of these studies were being done was of doubtful purity and the impurities may have been such as to cause some error. For example, buffer materials from the fermentation medium were often carried over to the final penicillin powder.

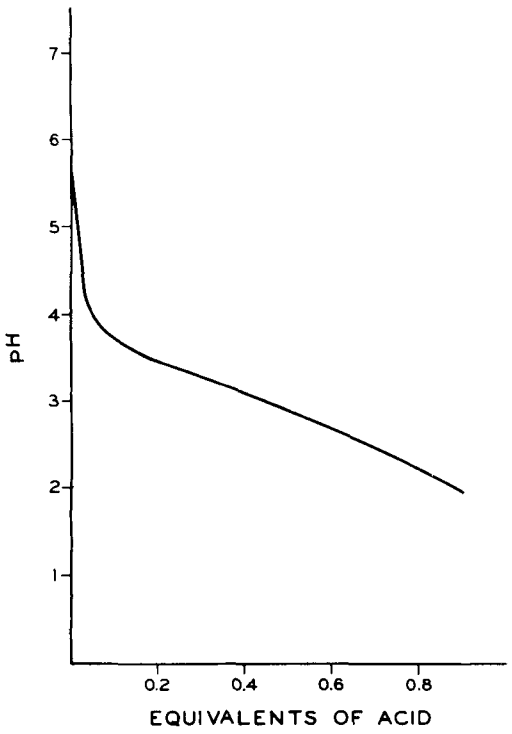
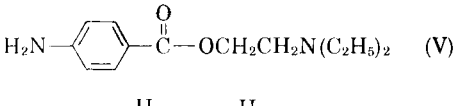


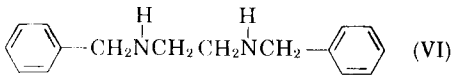
Fig. 3.—Titration curve of penicillin (potassium salt titrated with acid).

From what has already been said it is obvious that a solution of penicillin cannot, with present techniques, be made stable for more than about 2 weeks even at refrigerator temperatures. Partly for this reason, many sparingly soluble amine salts of penicillin were prepared. In these,

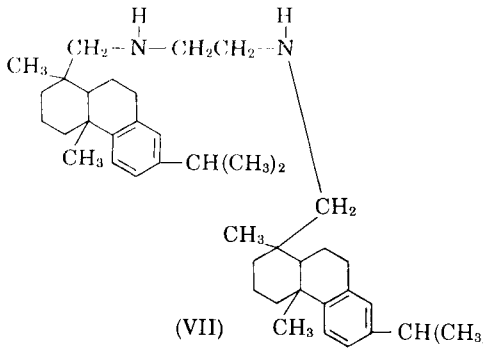
the protonated amine replaces the potassium or sodium of the soluble salt. Of these only a few have been marketed, *e.g.*, procaine (V), N,N′-dibenzylethylenediamine (VI), and N,N′-bis-(dehydroabietyl) ethylenediamine (VII). It was



(V)



(VI)



(VII)

found that suspensions of these salts in aqueous vehicles could be made stable for a year or more, allowing marketing of a "ready-made" penicillin product.

The stability of these salts in aqueous suspension is based mainly on their solubility and the rate of degradation of material in solution. A theoretical treatment of the solubility of these salts has been presented by Brunner (50, 51). He has derived equations by which one may calculate solubility as a function of pH, and pH of minimum solubility. These are based on mass-action law, a knowledge of the ionization constants of the amine and penicillin, and the solubility of the salt in pure water. The rate of loss of penicillin from a suspension in which the two phases are kept at equilibrium at constant temperature and pH will be pseudo zero order where the rate constant (k_0) will be the product of the first-order rate constant (k_1) for the material in solution, and the saturation concentration of penicillin under these conditions

$$\frac{-d(\text{Pen})}{dt} = k_0; \quad k_0 = k_1(\text{Pen})_{\text{sat.}}$$

Since the salt in solution is dissociated, or at least partly dissociated, some suppression of solubility may be achieved by the common ion effect. This has been demonstrated by Swintosky, *et al.* (52), with procaine penicillin by addition of procaine hydrochloride. These workers further reduced the solubility of the penicillin salt by the addition of salts and sorbitol to the vehicle. The rates of degradation of saturated solutions of procaine penicillin in this vehicle at several temperatures, as well as solubility as a function of temperature, were also measured (53). From these data predictions of long range stability of the product were made. These agreed fairly well with experimental values despite many assumptions made in the calculations.

While amine salts offered a means of stabilizing liquid penicillin preparations, generally their low solubility led to reduced penicillin serum levels after oral administration, as compared with those observed after oral administration of a soluble penicillin salt. It was demonstrated (54) that a stable oral liquid preparation of potassium penicillin could be made by suspending the salt in an edible, modified coconut oil. This preparation provided penicillin serum levels similar to those achieved with previously available preparations of the potassium salt.

Slawinska (55) showed that ultrasonic waves had a deleterious effect on stability of penicillin in aqueous solution, but not in paraffin suspension.

Stability in Ointments.—As is the case with powders and tablets, the stability of penicillin in ointment bases depends mostly on water content (56–58). In anhydrous bases the penicillin is relatively stable (59), whereas in emulsion bases the opposite is true. In Carbowax bases penicillin is also quite unstable (60, 61). Peroxides also appear to have a deleterious effect on penicillin stability. It was found that penicillin ointments made with peroxide-bleached bases lose about 30% potency in 7 months (62). If the peroxide value of petrolatum is kept below 0.06%, stability will be much greater (63). Procaine penicillin ointment made with a base consisting of peanut oil, beeswax, and petrolatum was stable for 4 years at room temperature (64).

INCOMPATIBILITIES OF PENICILLIN

Table I summarizes the reported incompatibilities of penicillin together with the appropriate literature references. As previously noted, penicillin is incompatible with acid and alkali and, in one sense, even with water. Any material which, when mixed with penicillin in solution, will change the pH away from the neutral region will be incompatible.

Generally alcohols, glycols, polyglycols, glycerin, and some sugars appear to react with

TABLE I.—INCOMPATIBILITIES OF PENICILLIN G

Type Compound	Reference
Oxidized cellulose	65
Chlorocresol	66
Glycerin and glycols	66–71
Heavy metals	72–76
Amines	77–80
Resorcinol	81
Zinc oxide	81, 82
Vitamin B_1	81
Procaine	81
Ephedrine	81, 83, 84
Iodine and iodides	81
Alcohols	71, 81, 85
Oxidizing agents	76, 79
Rubber tubing	86, 87
Thiols	88–92
Sugars	93, 94
Acids	82, 95
Aminoacridine hydrochloride	71
Thimerosal	71
Certain flavors	96

penicillin, probably by esterification of the potential acid group of the β-lactam, thereby forming biologically inactive penicilloates. Thiols act in a similar manner, although thiosulfate and metabisulfite have no deleterious effect (97). It has been reported, however, that penicillin is rapidly inactivated by sulfite and bisulfite (98). There was no sulfite found in the product and 82% of the original sulfite was recovered, indicating that some catalytic pathway is involved. Certain types of rubber tubing inactivate penicillin due to the presence of mercaptans from the vulcanization process (86, 87). Traces of heavy metals catalyze the hydrolysis and alcoholysis of penicillin. While the mechanism of this catalysis is not understood, it appears to involve the sulfur of the thiazolidine ring. Oxidizing agents also react with penicillin but the mechanism also is not known. The loss in activity might be caused by opening of the thiazolidine ring and oxidation of the sulfur to a sulfonic acid (penicillaminic acid) (76).

Amines may be incompatible with penicillin in two ways: precipitation of a salt if the latter is insoluble and/or reaction to form a penicilloamide.

STABILIZATION OF PENICILLIN

From the picture presented thus far, certain generalizations may be made concerning means of stabilization of penicillin in pharmaceutical preparations.

With solid dosage forms and ointments, the presence of water should be avoided to the maximum extent possible. This includes processing of materials during manufacture. It has been shown that dehydrating agents improve the stability of ointments containing up to 0.1% water (99).

The most important single factor in liquid formulations is pH control. Products made for solution will have maximum stability when pH is kept at about 6.5 by a buffer of sufficient capacity to maintain that pH throughout the useful life of the product.

With suspensions of amine salts, both solubility and rate of degradation are functions of pH, as shown in Fig. 4. The pH of optimum stability will be that at which the product of solubility and rate is a minimum. In addition, anything which reduces the solubility of the salt will aid stability, *e.g.*, addition of a soluble salt of the amine, salting out, etc. As a corollary, any substance which tends to solubilize the salt should be avoided in formulation. Since penicillin is known to react with certain amines, those chosen for preparation of insoluble salts should be unreactive toward the drug.

Claims have been made that several compounds "stabilize" penicillin in solution. Hobbs, for example, claimed that hexamine improved the stability of sodium penicillin G in solution (100). To the authors' knowledge this has not been refuted. When hexamine was added to a suspension of procaine penicillin, however, stability was decreased. This was due to some solubilization of the salt by the hexamine (101).

A German patent (102) claims the stabilization of penicillin solutions with soluble salts of 8-chlorotheophylline. The inventors claim a complex is formed in which the β-lactam is protected from hydrolysis. The preparation of this complex is given in a recent publication

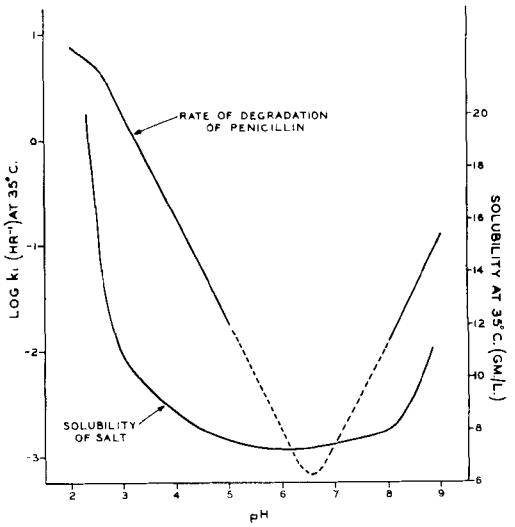


Fig. 4.—Solubility of procaine penicillin G and rate of degradation of penicillin G as functions of pH.

(103). Since the 8-chlorotheophylline is acidic, it is possible that this may be another case of a buffer effect causing stabilization. 8-Chlorotheophylline was ineffective as a stabilizer for phenethicillin in buffered solutions at pH 6.5 (104).

Aminopyrine (105) and sodium hexametaphosphate (76) also appeared to stabilize penicillin in solution, but again the comparisons were made with unbuffered solutions.

Paolini, *et al.* (106), have presented evidence to show that sodium sulfamethoxypyridazine "exerts a protective action against degradation of penicillin G sodium in aqueous solution at pH greater than 7." Their experiments however, were conducted in such a manner that pH dropped during each run, and this could have a significant effect on rate of hydrolysis. This is especially true above pH 7.5 where the rate becomes very highly pH dependent. Further work will be required to establish definitely whatever utility sulfonamides may have as stabilizers of penicillin.

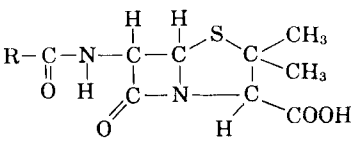
NEWER PENICILLINS

This section will compare the stability of the newer penicillins with penicillin G. For purposes of convenience penicillin V is included in this group although it should not be categorized with the synthetic penicillins. The chemical structures and nomenclature of these compounds are shown in Table II.

Acid Stability.—From a therapeutic standpoint, stability of penicillins to acid is quite important since it may be a limiting factor in oral effectiveness of the drugs. Table III gives the approximate half-life, at pH 1.3 and 35°, of several penicillins for which data are available. The most stable of these is ampicillin which has been recently marketed in Great Britain, the half-life being about 200 times that of penicillin G. This has been attributed to the electron attraction of the positively charged amino group which might be expected to hinder the electron displacements initiating rearrangement to the penillic acid (111). The authors also found a qualitative correlation between pKa of the side chain acids and acid stability of the penicillins. This should be useful in predicting acid stability of new penicillins, at least within a given structural group.

Stability in Pharmaceuticals.—As was the case with penicillin G, none of the newer penicillins is sufficiently stable in aqueous solution to

TABLE II.—NEWER PENICILLINS



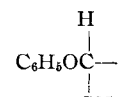
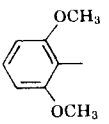
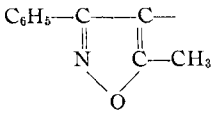
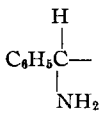
R	Nonproprietary Name	Chemical Name	Trade Name
$C_6H_5OCH_2$—	Penicillin V	Phenoxymethyl-penicillin	V-Cillin, Lilly Pen-Vee, Wyeth
	Phenethicillin	α-Phenoxyethyl-penicillin (107)	Syncillin, Bristol Darcil, Wyeth Chemipen, Squibb Alpen, Schering Maxipen, Pfizer Rocillin, Rowell Semopen, Massengill Dramcillin-S, White
	Methicillin	2,6-Dimethoxy-phenylpenicillin (108)	Staphcillin, Bristol Dimocillin, Squibb
	Oxacillin	3-Phenyl-5-methyl-4-isoxazolylpenicillin (109)	Prostaphlin, Bristol Resistopen, Squibb
	Ampicillin	α-Aminobenzyl-penicillin (110)	Penbritin, Beecham Laboratories (Great Britian)

TABLE III.—ACID STABILITY OF PENICILLINS

Penicillin	Half-life, min.[a]	Reference
"G"	3.5	111
"V"	160	111
Phenethicillin	68[b]	15
Methicillin	2.3	112
α-Methoxybenzyl	77	111
α-Chlorobenzyl	300	111
Ampicillin	660	111
Oxacillin	160	112

[a] pH 1.3, 35°, in 50% alcohol. [b] In aqueous solution (no alcohol present).

be marketed as such. The rate of degradation of phenethicillin in aqueous solution as a function of pH and temperature was investigated and found to follow the same general pattern as penicillin G, although the rates were slower (15). The pH of maximum stability was 6.5 and secondary phosphate ion was found to catalyze the loss of activity. Based on these data, a powder for reconstitution by the pharmacist at the time of dispensing was formulated. This lost only about 1% of its penicillin potency when reconstituted and stored for 2 weeks at refrigerator temperature (113). Phenethicillin in solution was studied in combination with 77 common liquid prescription preparations, most of which had no effect on stability for 3 days at 4° (113). Those which had the greatest effect were the products which changed the pH outside the range for maximum stability.

Methicillin is relatively unstable in aqueous solution. It has been reported that solutions kept at room temperature should be used within 12 hours of preparation and those stored at 4° within 24 hours (114). Work done in our own laboratories indicates that methicillin buffered with sodium citrate has much greater stability in solution.

Oxacillin is stable for about 24 hours at room temperature in aqueous solution buffered with sodium citrate (115).

Ampicillin, at neutral pH and room temperature, maintained 80% of its activity for 1 week (116). Whereas one would suspect that the other newer penicillins would show a dependence of rate of loss of activity on pH similar to that of penicillin G, one might also speculate that ampicillin might be different because of its zwitterionic character at almost neutral pH. From the reports available to date it appears to be the most stable penicillin available, in both acid and neutral solution.

Stability to Penicillinase.—It has been shown that the Michaelis constants for the catalysis of hydrolysis of these penicillins by staphylococcal penicillinase is in the order expected from previous experience with these drugs in treatment of infections caused by staphylococci resistant to penicillin G (117). That is, the greatest affinity for the enzyme is shown by penicillin G and the least by methicillin and oxacillin, the others being intermediate. Ampicillin is rapidly inactivated by penicillinase (112) but kinetic data are not available.

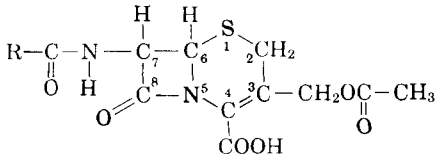
FUTURE PENICILLINS

Now that the means of making almost any penicillin is available, one may speculate on what the future may bring in this field. This might be done in terms of the properties desired in the ideal penicillin: (*a*) it should be active against a broad spectrum of microorganisms, including possibly fungi and viruses; (*b*) it should be sufficiently acid-stable so that it will not be destroyed in the stomach; (*c*) it should be rapidly and completely absorbed from all sites in the gastrointestinal tract, and slowly excreted; (*d*) it should not be strongly bound by serum protein; (*e*) it should be stable in aqueous solution; (*f*) it should be resistant to attack by penicillinase and/or other enzymes; (*g*) it should be nonallergenic; (*h*) it should be nontoxic.

Each of the newer penicillins has only two or three of these properties and, indeed, it may turn out to be impossible to make a single penicillin with all of them. Nevertheless, these criteria point the way for future research.

With regard to stability, it may be possible to make penicillins which will be stable in solution. In order to design such molecules further insight into the mechanisms of inactivation and the effects of structural changes upon these should be gained.

One group of compounds similar to the penicillins, but appearing to have much greater stability, are the cephalosporins. These are naturally occurring compounds which are derivatives of 7-aminocephalosporanic acid (VIII).



(VIII)

One of these, cephalosporin C

(R = H_3N^+—CH(—OOC)—$CH_2CH_2CH_2$—)

was found by Abraham and Newton (118) to inhibit competitively the hydrolysis of penicillin G by

penicillinase. These authors also isolated 7-aminocephalosporanic acid in very small quantities and acylated it with phenylacetyl chloride to obtain the derivative corresponding to penicillin G (119). This compound was several hundred times more active than cephalosporin C and yet resistant to hydrolysis by penicillinase. Apparently difficulties have been encountered which have prevented commercial production of these compounds thus far.

One other area which requires further exploration is the mechanism of action of penicillinase and the structural features of its active sites. This knowledge may enable us to design inhibitors of the enzyme.

SUMMARY

A survey of knowledge to date on stability of penicillins has been presented, including acid stability, stability in pharmaceutical preparations, and stability to the enzyme penicillinase. Incompatibilities and possible "stabilizers" were also discussed.

The side chain structure of the various penicillins endows each with different stability characteristics, although none available at present is sufficiently stable to allow marketing in solution.

Despite the great improvements in penicillins which have been made in the past 3 years, there is still much room for further work. With current ability to make structural modifications relatively easily, it is expected that even better penicillins will be seen in the future.

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Research Articles

Availability of Ionic Iron from Iron Chelates

By WINTHROP E. LANGE, ALFRED G. BARNET†, and WILLIAM O. FOYE

Several commercial hematinic preparations have been tested for their ability to liberate ionic iron at different conditions of pH and in the presence of simulated gastric and intestinal fluids. A chromatographic method was employed in which both Fe(II) and Fe(III) ions were measured by means of absorbance determinations. It was concluded that chelated, or complexed, iron is carried through the gastrointestinal tract with less loss and lower toxicity than is ionic iron.

Some confusion exists regarding the requisites for the state of iron necessary for therapeutic use as a hematinic. Brading, *et al.* (1), for instance, have reported that more Fe(III) than Fe(II) was absorbed by rats fed inorganic Fe^{59}, and that the distribution in the tissues did not depend on either dose or valency of iron. Hartwig, *et al.* (2), also found that $Fe^{59}Cl_3$ was incorporated into new erythrocytes to a significantly greater degree than was Fe^{59}(II) citrate. Elsewhere it has been stated that only ferrous iron can be absorbed and that the ferric form must first be reduced before it can enter the gastrointestinal mucosa (3).

The advantages of chelated *vs.* inorganic iron for therapeutic use also appear to be in question. Franklin, *et al.* (4), claimed that chelation of iron minimized its toxicity and did not impair its hemopoietic response in humans. A difference in effectiveness of iron chelate preparations has been noted (5), however, which is evidently due to relative ease of liberation of ionic iron from iron chelates. Injectable ferric ammonium citrate, for instance, gave low hemoglobin levels and erythrocyte counts in weanling pigs in comparison with injectable iron-dextran, oral iron in

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