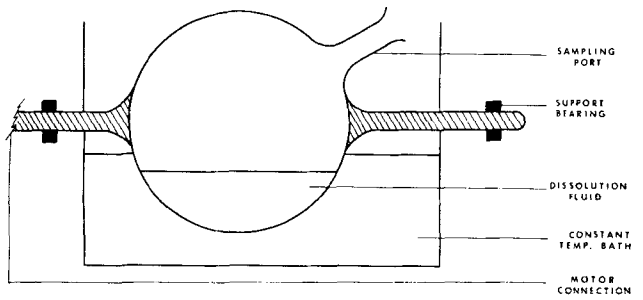

Figure 1—*Schematic diagram of dissolution apparatus. The flask has a diameter of about 16 cm. (6.5 in.)*

does not enter the port, regardless of the position of the flask as it proceeds through a revolution. These measures prevent the accumulation of undissolved solid in the port. The port is stoppered to prevent loss of the dissolution medium to the water bath. A review of the literature on dissolution methodology suggests that this method is unique, although the hydrodynamics of the present system may be similar to that found in the apparatus described by Ferrari and Khoury (4) or in the apparatus utilized by Simoons (5).

The dissolution rate of aspirin from three commercially available dosage forms—conventional tablets, buffered tablets, and timed-release tablets—was determined at 37° and 1.2 r.p.m. in 0.1 *N* HCl. Samples of the dissolution fluid were taken at frequent intervals by means of a filter pipet. The samples were then hydrolyzed and assayed spectrophotometrically at 302.5 mμ for salicylic acid. The dissolution data on each dosage form were compared with percent absorbed–time data on the same dosage forms in man from the literature (2, 6). The correlation is shown in Fig. 2 which is a plot of percent absorbed to time *T versus* percent dissolved *in vitro* at time *T*. Regression lines, calculated by the method of least squares, using all data were as follows:

$$\text{percent absorbed} = 0.79\,(\text{percent dissolved}) + 2.44 \quad \text{(Eq. 1)}$$

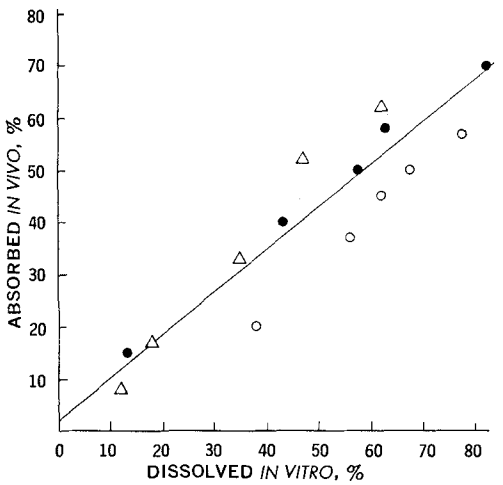

Figure 2—*Plot of percent of dose of aspirin absorbed to time* T *after drug administration* versus *percent dissolved* in vitro *at time* T. *Key:* ○, *conventional tablets;* ●, *buffered tablets, and* △, *timed-release tablets.*

$$\text{percent dissolved} = 1.08\,(\text{percent absorbed}) + 4.48 \quad \text{(Eq. 2)}$$

The solid line in Fig. 2 represents Eq. 1. Using the method described by Mather (7) for interclass correlation where both variables are normally distributed, the correlation coefficient was found to be 0.92 corresponding to $p < 0.001$.

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MILO GIBALDI
HOWARD WEINTRAUB*

Department of Pharmaceutics,
School of Pharmacy,
State University of New York at Buffalo,
Buffalo, NY 14214

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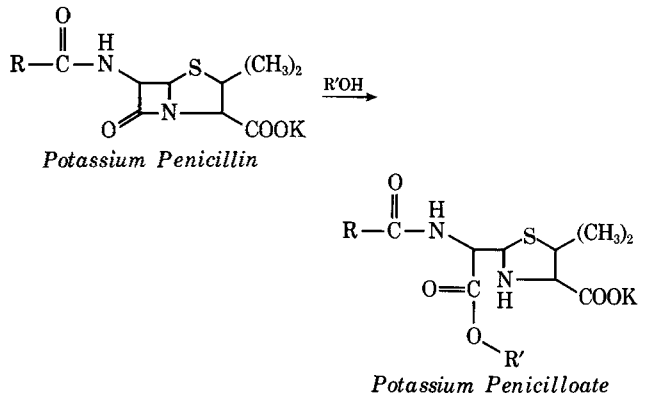
Penicillin Stability to Alcohols

Keyphrases □ Penicillin G, potassium, stability—alcoholic solution □ Stability—alcoholic potassium penicillin G solution

Sir:

There is considerable confusion in the literature with regard to the stability of penicillin solutions in the presence of alcohols. Several textbooks and other published works state that penicillin solutions are inactivated by alcohols and glycerol (1–14), and this statement is also found in the most recent volume of the *United States Pharmacopeia* under the monograph for Potassium Penicillin G (15). Perhaps because of this statement, at least one textbook on microbiology cautions that patients with venereal disease being treated with penicillin should avoid an intake of alcoholic beverages (16). Historically, one can trace the probable origin of the belief that penicillin is inactivated by alcohols to a report by Abraham and Chain (17) who reported in 1942 that alkali salts of penicillin rapidly lose their biological activity when dissolved in primary alcohols. Presumably, alcoholysis proceeds with the ultimate formation of the appropriate inactive monoester of penicilloic acid (Scheme I).

However, in 1948, Chain *et al.* (18) conclusively demonstrated that this decomposition of penicillin



Scheme I—Alcoholysis of potassium penicillin to inactive potassium penicilloate

salts in methanol occurred only in the presence of zinc, copper, or tin, which were contaminants in the alcohol that was used to demonstrate deactivation of penicillin as previously reported (17). They postulated that rather than a direct alcoholytic fission of the penicillin β-lactam ring, alcoholysis probably resulted through an intermediate oxazolone (penicillenic acid), formed by attack of the metal ion on the sulfur of the molecule (18). This inactivation of penicillin by metal-contaminated alcohol was retarded by dimercaptopropanol (18). These findings were later confirmed by Parnaby, who pointed out that alcoholic penicillin solutions were as stable as aqueous penicillin solutions (19).

Brodersen (20), while studying the kinetics of penicillin–penicillinase reactions, found that ethanol could be used to halt the enzymatic reaction without inactivating residual penicillin. At about the same time, other workers were using ethanol to eluate adsorbed penicillin from charcoal chromatographic columns in relatively good yields, which offers additional evidence that alcohols do not adversely affect penicillin stability (21). Indeed, penicillin has even been used in the past as a preservative of beer (22).

Finally, one can point to Fleming's original report on penicillin in which he indicates that penicillin activity could be extracted into ethanol from concentrated fermentation media (23).

Clearly, full consideration of the meaning of these early studies has not been given, and the concept of penicillin–alcohol "incompatibility" remains to this day. Curiously enough, with the voluminous literature available on all aspects of penicillins, a review by Doyle and Nayler (24) is the only report that we have found that clearly points out that penicillins are stable in the presence of alcohols.

Preliminary studies in our laboratory indicated that potassium penicillin G, following incubation with various concentrations of ethanol contained in nutrient broth, human serum, and human plasma, indeed continued to exhibit antibacterial activity against a known penicillin-sensitive strain of *Staphylococcus aureus*. Similar *in vitro* activity of penicillin in the presence of benzyl alcohol was demonstrated as well.

Thus, to derive more quantitative data and to establish that penicillin is stable in the presence of alcohols, we initiated a series of simple confirmatory experiments

Table I—Assay Results for Residual Potassium Penicillin G

Day after Preparation of Solution	Potassium Penicillin G Remaining, %				
	Predicted[a]	Solution A[b]	Solution B[c]	Solution C[d]	Solution D[e]
1	98.3	99.0	97.0	99.0	96.9
2	96.7	96.3	94.0	97.4	97.1
3	95.0	95.5	96.3	93.5	94.4
4	93.4	92.0	94.9	90.0	89.8
5	91.0	92.0	90.0	92.5	88.4
6	90.0	91.8	89.0	89.2	85.0
7	88.0	88.5	87.0	88.0	85.2
8	86.0	84.6	87.0	84.0	83.6
9	84.0	86.4	85.2	84.0	83.2
10	83.0	85.1	84.1	85.0	82.0

[a] Calculated from published tables (20). [b] Potassium penicillin G control solution. [c] 40% ethanolic potassium penicillin G solution. [d] 70% ethanolic potassium penicillin G solution. [e] 0.5% benzyl alcohol potassium penicillin G solution.

on the subject. Ethanol and benzyl alcohol were utilized, the latter because sterile distilled water containing benzyl alcohol as a preservative is used in many hospitals as a diluent for the preparation of solutions of penicillin for parenteral administration.

Experimental—*Materials*—The penicillin used was commercially available potassium penicillin G. All solutions used in this study were prepared with a 1% phosphate buffer pH 6.0 (2.0 g. dibasic potassium phosphate, 8.0 g. monobasic potassium phosphate, and sufficient distilled water to make 1000 ml.) to contain a final concentration of 2000 u./ml. of potassium penicillin G. Subsequent sterilization of these solutions was done by Millipore filtration (0.22-μ pore size), after which they were stored at room temperature in previously sterilized glass flasks fitted with screw caps.

Assay Procedure—The method used to determine residual penicillin was an iodometric titration adapted from Alicino (25) and Mundell *et al.* (26) as described by Grove and Randall (27). At predetermined times (Table I), 2-ml. aliquots from each of the test solutions were aseptically removed and assayed for total penicillin. Each assay was done in duplicate, and the results were averaged. As a precautionary measure to detect accidental contamination by microorganisms, each day a small amount from each penicillin test solution was diluted with sterile saline and the resulting mixture was passed through a Millipore filter apparatus. Three further rinses with saline through the filter served to remove all residual penicillin, after which the membrane filter was removed, incubated on a suitable solid medium according to standard procedures, and observed for evidence of bacterial or fungal growth. No contamination was noted for all solutions throughout the course of this study.

Preparation of Penicillin Test Solutions—Penicillin Control (Solution A)—A sterile and buffered penicillin solution was prepared as described under *Materials*.

Ethanol Penicillin Solutions (Solutions B and C)—Two separate penicillin solutions were prepared as directed for Solution A, except that a portion of the water in each solution was replaced with 95% ethanol to furnish final concentrations of 40% (Solution B) and 70% (Solution C) ethanol, respectively.

Benzyl Alcohol Penicillin Solution (Solution D)—A sterile aqueous solution of benzyl alcohol (0.5%) was used as the vehicle to prepare the buffered penicillin solution.

Results and Discussion—The data from Table I show that the amounts of potassium penicillin G remaining in the control solution (Solution A) at specific times after the start of the experiment coincide rather well with predicted values which were calculated from published tables (20). Furthermore, it is noteworthy that the observed residual penicillin concentrations in the ethanol (Solutions B and C) as well as the benzyl alcohol (Solution D) solutions compared favorably with those of the penicillin control (Solution A).

Thus, these experiments have shown that potassium penicillin G can be mixed with ethanol solutions of concentrations as high as 70% for up to 10 days, with no greater loss than penicillin in aqueous solution. In a similar manner, it has been shown that benzyl alcohol, at a concentration of 0.5%, will not cause a decrease in stability of potassium penicillin G when in contact with the antibiotic for periods up to 10 days.

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ALVIN B. SEGELMAN
NORMAN R. FARNSWORTH
Department of Pharmacognosy
School of Pharmacy
University of Pittsburgh
Pittsburgh, PA 15213

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Books

REVIEWS

Experimental Methods in Pharmacology. By CHARLES O. WARD. St. John's University Press, Jamaica, NY 11432, 1969. 119 pp. 15 x 23 cm.

This small book briefly describes 62 experiments under 18 different chapter headings. Some of the subjects covered are local anesthetics, psychotropic drugs, anticonvulsants, muscle relaxants, topical drugs, antihistamines, and diuretics. Most of the experiments described are time-tested. Unfortunately the book is not of general value even though it is well suited to the author's laboratory. The descriptions of the experiments are very short and in many cases not too informative. Many numbers are missing for dosages and stimulus parameters; no statement of the intent of the blank spaces is given. This reviewer presumes that the student is to furnish the numbers by reference to the literature.

In some cases the experiments are so inadequately described as to be seriously misleading. An example is the experiment on measuring blood flow with a rotameter. The obvious requirement that flow measurements must have simultaneous pressure measurements to have physiological meaning is ignored. In addition the use of the carotid artery is questionable because of the parallel flow (unmeasured) through three other large arteries.

The book is marred by many typographical errors; some of these are quite humorous, such as untravenously and cammula. Dosages are given in variable units; cc. of solution per kgm. or by weight per kgm. Anesthesia seems to have been omitted in some experiments in which it seems indicated. In an experiment in which painful stimuli are applied to a guinea pig, no description of the animal's response is given.

This edition of "Experimental Methods in Pharmacology" cannot be recommended for general use. The ideas behind it are excellent, and a revision based on discussions with other pharmacology teachers would be a welcome addition.

Reviewed by R. P. Ahlquist
Medical College of Georgia
Augusta, GA 30902 ■