New Concept of Competitive Inhibition of the Renal Tubular Excretion of Penicillin

KARL H. BEYER,[1] *Department of Pharmacology, Sharp & Dohme, Inc., Glenolden, Pennsylvania*

It has been established that penicillin is excreted by way of the renal tubules in addition to glomerular filtration (*1*). Its over-all clearance at all plasma concentrations studied to date appears to be equivalent to renal plasma flow, as measured directly or by p-aminohippuric acid, the extraction ratio of which is reported to be 0.88:0.93 (*9*). Where a normal relationship exists between glomerular filtration and renal plasma flow, the fraction of penicillin filtered at the glomeruli represents in man about 20 per cent of the total amount excreted per unit time. Conversely, at customary blood levels, about 80 per cent of the penicillin excreted is eliminated by way of the renal tubules. Thus, the suppression of penicillin excretion by the tubules could be expected to effect a considerably higher plasma concentration for a longer period of time than normally would obtain following a given dose of penicillin.

This reversible inhibition of the excretion of penicillin by the tubular epithelium has been effected by the administration of diodrast (*8*) or PAH (p-aminohippurate) (*2*). These compounds are excreted by the same tubular transport mechanism and as rapidly as penicillin. Hence, it is possible, by maintaining a high plasma concentration of either compound, to saturate functionally that mechanism and thus suppress the tubular elimination of penicillin on what loosely may be considered a "mass action" basis. This principle has been applied successfully in therapy (*5*), but the very great amount of PAH needed to produce this effect seriously limits its applicability from a practical standpoint.

Acknowledging these limitations requiring intravenous administration and very large dosages of PAH with penicillin, a new approach was sought which resulted in the concept involving the application of the compound described herein. This compound appears to combine the advantages of high and sustained blood levels of penicillin administered orally or parenterally at dosages that are feasible.

The purpose of this report is to present briefly the broad aspects of this concept without detailed qualification or substantiation of each point. Laboratory and clinical substantiation will be submitted in detail in a forthcoming series of publications.

Excretion by the renal tubules is a remarkably selective process. The transport mechanism for the excretion of penicillin, like other differentiating mechanisms for glucose and amino acid reabsorption, is dependent on the viability of the tubular epithelium. Although without precedent, it seemed desirable to inhibit reversibly and selectively the transport mechanism for the excretion of penicillin without necessarily abolishing other transport systems or impairing the vitality of the cell as a whole. If this could be done, the remaining pathway for penicillin excretion would be glomerular filtration, which would represent only about 20 per cent of the normal rate of penicillin elimination.

While in effect there may be a number of such seemingly discrete systems whose functional orientations are directionally opposite within the cell (excretion-absorption), the multiple components of a single transport mechanism need not be unique, either descriptively or functionally, to that system that gains singularity only through some definitive component or components. Since most metabolic processes are enzymatic, it seemed possible to inhibit selectively such a fundamental or definitive enzymatic component of an otherwise complex process and so halt the particular system involved in the excretion of penicillin.

The competitive inhibition of the enzymatic alteration of one substrate by another which has an affinity for, but is refractory to, the action of an enzyme or enzyme system occurs *in vitro*. Examples of such *in vitro* substrate competition are the inhibition by malonate of succinate oxidation by the succinoxidase system (*7*), and the inhibition by ephedrine of tyramine deamination by amine oxidase (*3*). Moreover, the affinity of the competitive substrates appears to be for the definitive enzyme, succinic dehydrogenase (*6*) or amine oxidase (*4*), in each complex system. These are reversible inhibitions wherein the integrity of the enzyme system has not been violated inalterably.

TABLE 1

PROTOCOL OF AN EXPERIMENT ILLUSTRATING THE INHIBITORY EFFECT OF 4'-CARBOXYPHENYLMETHANESULFONANILIDE ON PENICILLIN CLEARANCE

Time (hr.:min.)	Urine vol. (cc./min.)	Penicillin		Creatinine clearance (cc./min.)	Filtration fraction
		Plasma conc. (units/cc.)	Clearance (cc./min.)		
Dog 240					
Control phase	*Penicillin—Priming* dose: 15,200 units, i.v. Maintenance dose: 156 units/min. in 5% glucose, 3 cc./min., i.v.				
0:35	3.1	0.68	278.1	75.2	0.27
0:45	3.3	0.58	212.8	63.6	0.30
Drug phase	*Penicillin*—Maintenance dose: 136.5 units/min. at 3 cc./min., i.v. *Drug*—Priming dose: 25 mg./kg., i.v. Maintenance dose: 30 mg./kg./hr., i.v.				
1:30	3.5	0.79	51.9	60.3	1.16
1:40	3.6	0.83	57.2	59.5	1.04

Transposing into terms of renal physiology, if it were possible to find a compound which would inhibit the excretion of an agent by the tubules on the basis of specific enzymatic substrate competition, it should have the following essential chemical and pharmacodynamic characteristics: (1) It should have an affinity for the particular enzyme system that characterizes the selectivity of the over-all tubular mechanism for penicillin excretion. (2) If it is sufficiently different to be refractory to the action of that system, its renal elimination should be limited essentially to glomerular filtration. (3) The inhibition of penicillin excretion should be maximal at relatively low plasma concentrations, as compared to the type of mass action effect induced by PAH. (4) It should not alter the function of other transport mechanisms either selectively

[1] The data presented here were obtained through the cooperation of Drs. W. F. Verwey and A. K. Miller, of the Department of Bacteriology, and Mr. H. F. Russo and Miss E. A. Patch, of the Department of Pharmacology.

or collectively at plasma concentrations sufficient to inhibit maximally the tubular excretion of penicillin. Effects produced at excessive elevations of blood levels of the compound sufficient to be toxic in the customary sense of the word are, of course, beyond the intent of this principle. (5) If the agent acts by competitive inhibition of an enzymatic reaction, in accordance with this concept, the process should be reversible. (6) Such a compound should not necessarily influence either renal blood flow or glomerular filtration rate. However, it would vitiate the use of PAH for the measurement of renal plasma flow or normal PAH_{Tm}, for PAH is excreted in a manner similar to that for penicillin. (7) The agent should not have a high order of systemic toxicity. (8) The compound need not necessarily influence any properties peculiar to penicillin, such as its bacteriostatic action, inactivation, etc. (9) It follows from (2) and (3) that the dosage of the compound would be quite practical. (10) It was anticipated that these properties might be contained in a compound or compounds having the additional advantage of oral efficacy at reasonable dosage.

Of the compounds synthesized for this research by the Department of Organic Chemistry of these Laboratories, 4′-carboxyphenylmethanesulfonanilide incorporates essentially the properties listed above.

In the experiment summarized in Table 1, penicillin was infused at a rate and in an amount that would permit a falling penicillin plasma concentration if the drug were not effective. Duplicate control penicillin and creatinine clearances were obtained. The drug then was injected as a priming and maintenance dose, its distribution in the body allowed to equilibrate, and additional penicillin clearances obtained. It may be seen that the tubular excretion of penicillin was completely suppressed, as indicated by the rising penicillin plasma concentration, decreased penicillin clearance, and increased filtration fraction.

Fig. 1 illustrates two experiments wherein dogs were administered 100,000 units of penicillin by stomach tube every 4 hours for 16 hours, including the 4-hour period wherein the control curves for penicillin plasma concentration were obtained. Immediately after the 4-hour control blood samples were taken, each dog was given 100,000 units of penicillin plus 60 mg./kg. of the drug by stomach tube, another curve for plasma concentration of penicillin being obtained over the next 4-hour period. It may be seen that in the control phase the maximal penicillin plasma concentrations were 0.8 and 1.2 units/cc. and at 2 hours the values were less than the lower limits of the Florey cup-plate assay, 0.24 units/cc. Following administration of drug and penicillin the maximal penicillin plasma concentrations were 2.85 and 3.6 units/cc. In one instance the peak occurred at 2 hours following oral administration. At 4 hours the plasma concentration was still somewhat above 0.5 units/cc.

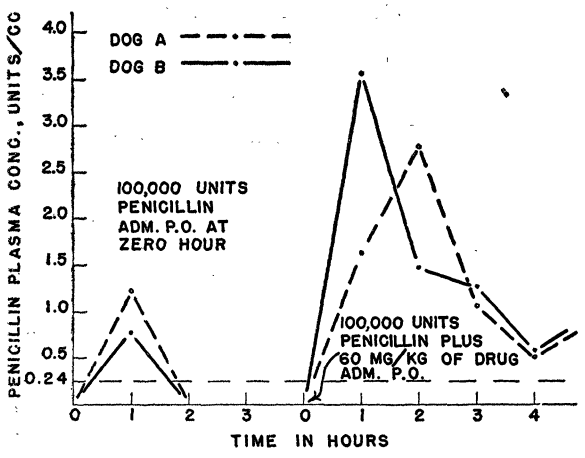

FIG. 1. The blood level response to equivalent oral dosages of penicillin alone and with the coadministration of 4′-carboxyphenylmethanesulfonanilide.

Summary. It has been found that the excretion of penicillin by a renal tubular transport mechanism could be physiologically inhibited reversibly. The basis for this effect is thought to be one of substrate competition between penicillin, which is excreted by the tubules, and 4′-carboxyphenylmethanesulfonanilide, which is essentially refractory to excretion by that transport mechanism.

References

1. BEYER, K. H., PETERS, L., WOODWARD, R., and VERWEY, W. F. *J. Pharmacol.*, 1944, **82**, 310; RANTZ, L. A., KIRBY, W. M. M., and RANDALL, E. *J. clin. Invest.*, 1944, **23**, 789.
2. BEYER, K. H., WOODWARD, R., PETERS, L., VERWEY, W. F., and MATTIS, P. A. *Science*, 1944, **100**, 107.
3. BLASCHKO, H., RICHTER, D., and SCHLOSSMAN, H. *Biochem. J.*, 1937, **31**, 2187.
4. GOVIER, W. M., GRELIS, M. E., YANZ, N. S., and BEYER, K. H. *J. Pharmacol.*, 1946, **87**, 149.
5. MOKOTOFF, R., BRAMS, W., KATZ, L. N., and HOWELL, K. M. *Amer. J. med. Sci.*, 1946, **211**, 395; AVERY, N. L., MAYER, O. B., and NELSON, R. C. *Ann. int. Med.*, 1946, **24**, 900; LOEWE, L., ROSENBLATT, P., and ALTURE-WEBER, E. *Amer. Heart J.*, 1946, **32**, 327.
6. QUASTEL, J. H., and WHEATLEY, A. H. M. *Biochem. J.*, 1931, **25**, 117.
7. QUASTEL, J. H., and WHETHAM, M. D. *Biochem. J.*, 1925, **19**, 520.
8. RAMMELKAMP, C. H., and BRADLEY, S. E. *Proc. Soc. exp. Biol. Med.*, 1943, **53**, 30.
9. WARREN, J. V., BRANNON, E. S., and MERRILL, A. J. *Science*, 1944, **100**, 108; BRADLEY, S. E. Personal communication.

Some Effects of O-Isopropyl N-Phenyl Carbamate Upon Cereals[1]

W. B. ENNIS, JR.

Camp Detrick, Frederick, Maryland

Most of the reports in the literature concerning the use of chemicals as plant-growth regulators indicate that application of such substances in some form to the aerial portions of plants is the most common practice. In the instance of O-isopropyl N-phenyl carbamate, however, greatest success appears to have been achieved through applications to the soil in a suitable diluent or carrier, and the results of applications in sprays to the plant itself have been inconclusive (*1*, *2*). To test further the herbicidal activity of sprays of this compound, several experiments have been carried out using oats and barley as test plants.

To insure thorough wetting of the leaves, young oat and barley plants were exposed by immersion of the aerial plant portions for intervals of 2–4 seconds up to 10 minutes in isopropyl alcohol-water solutions containing 500, 250, or 100

[1] These studies were conducted from March 1946 to August 1946 under the supervision of Dr. C. E. Minarik.