Renal Clearance of Pantothenic Acid in Man: Inhibition by Probenecid ('Benemid').* (20191)

WILLIAM P. BOGER, GILBERT M. BAYNE, JULINA GYLFE, AND LEMUEL D. WRIGHT.

From the Department of Research Therapeutics, Norristown State Hospital, Norristown, Pa. and Department of Microbiological Chemistry, Sharp and Dohme, West Point, Pa.

Probenecid has been shown to inhibit reversibly the renal tubular secretion of a number of organic acids, such as penicillin (1,2) phenolsulfonphthalein(3), and para-aminohippuric acid (PAH)(3,4). The plasma levels of para-aminosalicylic acid(5) and of the sulfonamides(6) also may be elevated by the coadministration of probenecid, but to a lesser degree than is the case with penicillin. The actual increase is small with PASA and sulfonamides and the mechanism by which this is accomplished has not been clearly demonstrated, but inhibiton of renal tubular secretion may be one of the mechanisms involved.

One of us (L.D.W.)(7) had previously studied the renal clearance of pantothenate in dogs, showing that with the 100-fold range of dosage employed, the clearance of pantothenate was not significantly greater than exogenous creatinine clearance. On the other hand, Roholt and Schmidt(8) in Sweden, who investigated pantothenate clearances in man over a wide range of plasma concentrations, reported pantothenate clearance to be significantly greater than inulin clearance at high plasma concentrations. At still higher plasma levels, a self-depression of pantothenate clearance or secretory Tm phenomenon was observed. This group has reported an inhibition of pantothenic acid clearance with carinamide.† Inasmuch as probenecid effects, in terms of a number of reference substances, have been shown to parallel those reported for carinamide, we were interested to see to what degree probenecid would inhibit the renal tubular secretion of pantothenic acid.

Methods. The pantothenic acid content of plasma and urine was determined by the microbiological method of Skeggs and Wright (9) and expressed in terms of calcium pantothenate. As a measure of glomerular filtration rate, urine and plasma specimens were analyzed by a modification of the Folin-Wu method(10) for endogenous materials that yield color with alkaline picrate. Endogenous chromogen clearance values are slightly lower than true creatinine clearances but Brod and Sirota indicate that chromogen clearance is a useful clinical test of filtration rate in man(11). The "creatinine" clearances referred to in this study are actually chromogen clearances. Since little, if any, chromogen is excreted by tubular secretion when plasma levels are in the normal range, the chromogen clearance may be regarded as an approximate measure of glomerular filtration rate even when probenecid is given concurrently. The plasma and urine PAH determinations were done by the method of Smith and associates(12). Our observations are presented in 2 sections, thereby conforming to the pattern of the investigation. The plan of study will be presented along with the results for each portion of the work.

Probenecid effect upon plasma pantothenate levels. In order to determine the loading dose required to attain plasma levels of pantothenate that would be associated with significant renal tubular secretion, sodium pantothenate was injected rapidly (2 to 3 minutes) by the intravenous route. Of the individuals presented in Fig. 1, L.B. received 500 mg and M.J. received 1000 mg of sodium pantothenate on each of two occasions: alone, and one hour after an intravenous injection of 2.0 g of probenecid. Comparison of the plasma values of pantothenate after the administration of pantothenate alone with those after pantothenate and probenecid at one, 2, and 3 hours after injection showed clearly that probenecid retarded the rate at which the

* Probenecid is the generic name for p-(di-n-propylsulfamyl)-benzoic acid, which is marketed under the trademark 'Benemid' by Sharp and Dohme, Inc.

† Carinamide is 4'-carboxyphenylmethanesulfonanilide, formerly marketed under trademark 'Staticin' by Sharp and Dohme, Inc., Philadelphia, Pa.

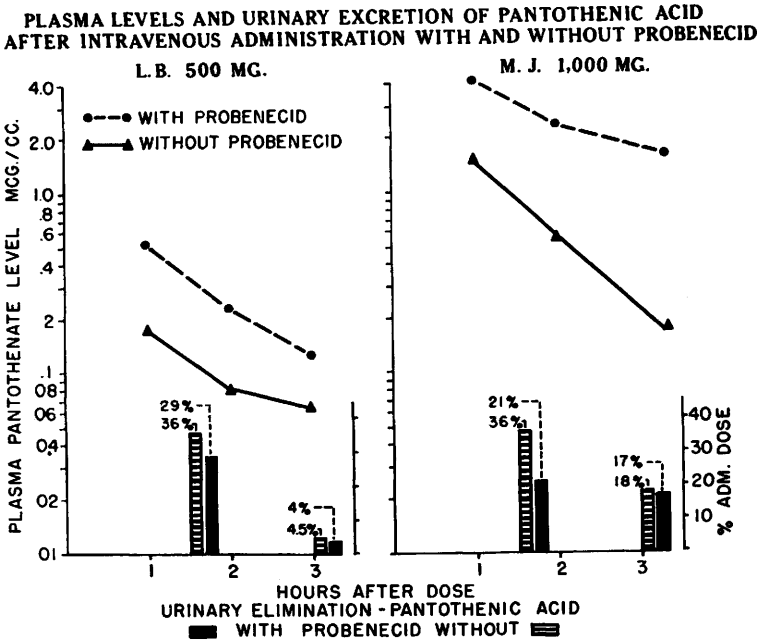

FIG. 1. Probenecid, 2.0 g intravenously, produced in two patients a 2- to 10-fold increase of pantothenate plasma concentrations over a 3-hr period after the intravenous administration of 500 and 1000 mg of sodium pantothenate respectively. Urinary pantothenate recoveries measured simultaneously showed a slight depression under the influence of probenecid.

plasma pantothenate levels declined. The urinary excretion of pantothenic acid was determined for the 0 to 1½ and the 1½ to 3 hour post-injection periods and it will be noted that the excretion after probenecid was less than when sodium pantothenate was given alone. With these indications that there was tubular secretion of pantothenic acid that could be inhibited by probenecid at these levels of plasma pantothenate, it seemed purposeful to perform formal clearance studies.

Clearance Studies. In one study simultaneous creatinine, pantothenate and PAH clearances were measured. PAH was given in an amount suitable for determining renal plasma flow. Patient R.R. fasted from midnight until 8 a.m., when she received 350 cc of water and Nembutal 1.5 grains. The patient was catheterized at 9:15 and a 20-minute control urine specimen was collected. Following this, a control blood sample was drawn and a priming injection of 500 mg of sodium pantothenate given. At the same time a sustaining infusion of sodium pantothenate and PAH in 5% dextrose in water was begun and a period of 46 minutes was allowed for equilibration. Throughout the study, pantothenate was infused at a rate of 0.157 mg/kg/min. and PAH at a rate of 0.09 mg/kg/min. Six blood specimens were obtained at appropriate intervals during the control and post-probenecid periods to allow construction of a curve for plasma levels from which the plasma values for each period might be calculated. After 3 control urinary collection periods a 2.0 g dose of probenecid was administered intravenously and 1.0 g was added to the infusion fluid. After 10 minutes was allowed for the establishment of the probenecid effect, urine was collected for 3 additional periods. Fig. 2 presents the observations that were made. In the control periods pantothenate clearance significantly exceeded creatinine clearance but did not approach PAH clearance. Under the influence of probenecid, pantothenate clearance approximated glomerular filtration rate. Probenecid also produced a significant diminution in PAH clearance but this clearance was not depressed to glomerular filtration rate. This degree of effect upon PAH clearance is in accord with that which we have described elsewhere(3)

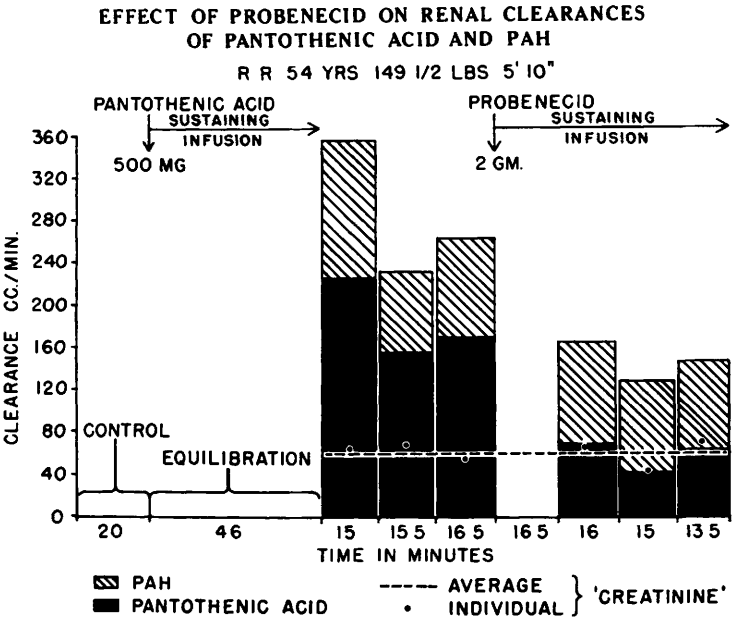

FIG. 2. Simultaneous measurement showed the normal pantothenate clearance to be intermediate between the PAH clearance and the creatinine clearance (glomerular filtration) rate. Under the influence of intravenously administered probenecid the clearance of pantothenate was depressed to creatinine clearance whereas PAH clearance was depressed only moderately.

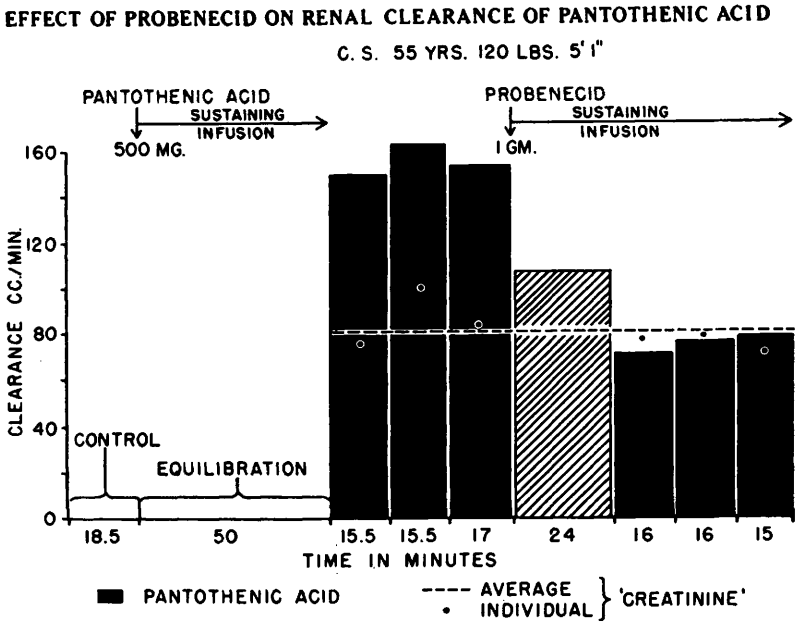

FIG. 3. Pantothenate renal clearance was measured in 3 control periods and found to exceed creatinine clearance (glomerular filtration) rate. Under the influence of intravenously administered probenecid the pantothenate clearance was depressed rapidly (within 24 min.) to a value approximating creatinine clearance rate.

and which has been confirmed by others(4). The probenecid plasma levels of 16 to 20 mg % that were maintained during this study were in excess of those required for maximum inhibition of tubular secretion of penicillin(2).

From this study it seemed reasonable to infer that pantothenic acid and PAH were probably handled by the same renal transport mechanism. Although it seemed unlikely that the small amounts of PAH administered to patient R.R. would significantly affect the pantothenate clearance, additional clearance studies were done in which the measurement of renal plasma flow with PAH was omitted. The interrelationship between substances being cleared has been previously noted(13).

The clearance study on patient C.S. was similar to that just described, the exceptions being the omission of PAH and the giving of a 1 g priming infusion of probenecid rather than 2 g. The same priming dose (500 mg) and the same rate of infusion of sodium pantothenate (0.157 mg/kg/min.) was employed.

Again (Fig. 3) the clearance of pantothenate was significantly greater than creatinine clearance during the control period but approximated creatinine clearance after probenecid. The hatched area represents the clearance for the post-probenecid equilibration period and indicates the rapidity with which probenecid exerts its effect.

Discussion. The studies of the renal clearance of pantothenic acid in dogs(7) demonstrated that the major portion, perhaps all, of the pantothenic acid present in normal plasma is in a nondiffusible form, and at low plasma concentrations this may complicate the calculation of clearance values. It was shown also that, when the plasma pantothenate level was elevated by intravenous pantothenate infusion, the added pantothenate was readily diffusible and was filtered at the glomerulus. Our patients had duplicate control values for plasma pantothenate that averaged 0.12 μg/cc (range of 0.08 to 0.21 μg). Pantothenate levels were not corrected by subtracting these control values for the calculation of pantothenate clearances. Even if the control pantothenate levels represented only nondiffusible material, as in the dog, the maximum error introduced would approximate 0.3%, since plasma concentrations of 30 to 50 μg/cc were maintained during these studies. We did not have available the technics for the study of the diffusibility of pantothenate when infused into man and our conclusions are drawn on the assumption that the findings of such a study would not alter the results qualitatively.

The foregoing observations indicate that pantothenic acid is another organic acid that can be actively secreted by the human renal tubule. Further, it seems likely that this is accomplished by the same renal transport mechanism that is involved in the tubular secretion of penicillin, PSP and PAH. Although it has not been demonstrated that any of these three latter substances will competitively inhibit the tubular secretion of pantothenate, it would be anticipated that PAH in quantity might do so in the same manner as it inhibits penicillin secretion(14). Two agents, carinamide and probenecid, have exhibited sufficient affinity for the transport system involved to inhibit reversibly the tubular secretion of all four of these reference substances. Carinamide was a safe and effective drug(15), but was impractical because of the large daily dose (18 to 24 g). Probenecid with a much greater affinity for the system involved (as judged by the smaller effective daily dose of 2 g) has been applied to the handling of a number of clinical circumstances.

The observation that probenecid will inhibit the tubular secretion of pantothenic acid has been made. The therapeutic implications of this observation are not apparent at this time.

Summary. 1. At plasma levels of 30 to 50 μg/cc, significant renal tubular secretion of pantothenic acid occurs. 2. It is probable that pantothenic acid is secreted by the same renal tubular transport mechanism involved in the transport of penicillin, phenolsulfonphthalein and PAH. 3. Probenecid can inhibit effectively the renal tubular secretion of pantothenic acid in man. 4. Probenecid enhances the plasma pantothenate levels obtained by the intravenous administration of sodium pantothenate in doses of 500 and 1000 mg.

We are indebted to Mr. P. W. Wilcox, Sharp and Dohme, Inc. for supplying us with intravenous preparations of sodium pantothenate and probenecid; to Dr. John Baer for the creatinine and PAH de-

terminations, and to Mrs. Charlotte A. Driscoll for the pantothenate assays.

1. Boger, W. P., Gallagher, M. E., Pitts, F. W., *J. Phila. Gen. Hosp.*, 1950, v1, 51.

2. Boger, W. P., Beatty, J. O., Pitts, F. W., Flippin, H. F., *Ann. Int. Med.*, 1950, v33, 18.

3. Boger, W. P., Matteucci, W. V., Schimmel, N. H., *Am. J. Med.*, 1951, v11, 517. (Am. Fed. Clin. Research Abst.)

4. Sirota, J. H., Yu, T. F., and Gutman, A. B., *J. Clin. Invest.*, 1952, v31, 692.

5. Boger, W. P., and Pitts, F. W., *Am. Rev. Tuberc.*, 1950, v61, 862.

6. Crosley, A. P., Bayne, G. M., Carfagno, S. C, and Boger, W. P., *J. Lab. and Clin. Med.*, 1952, v40, 730.

7. Wright, L. D., Beyer, K. H., Skeggs, H. R., Russo, H. F., and Patch, E. A., *Am. J. Physiol.*, 1946, v145, 663.

8. Roholt, K., and Schmidt, J., *Scandinavian J. Clin. and Lab. Inv.*, 1951, v3, 108.

9. Skeggs, H. R., and Wright, L. D., *J. Biol. Chem.*, 1944, v156, 21.

10. Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, v38, 81.

11. Brod, J., and Sirota, J. H., *J. Clin. Invest.*, 1948, v27, 645.

12. Smith, H., Finklestein, N., Aliminosa, L., Crawford, B., and Graber, M., *J. C. I.*, 1945, v24, 388.

13. Smith, H. W., Goldring, W., and Chasis, H., *J. C. I.*, 1938, v17, 263.

14. Beyer, K. H., Woodward, R. Peters, L., Verwey, W. F., and Mattis, P. A., *Science*, 1944, v100, 107.

15. Boger, W. P., and Crosson, J. W., *Am. J. Med*, 1950, v9, 35.

Received March 11, 1953. P.S.E.B.M., 1953, v82.

Conversion of Adrenocortical Hormones to More Polar Compounds by Adrenal Slices.* (20192)

ROBERT HAYNES, KENNETH SAVARD, AND RALPH I. DORFMAN.

From Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

During the course of studies involving adrenal slice incubations, it was noted that extracts prepared from the slices after incubation sometimes contained 3 compounds which migrated very slowly on paper chromatograms. At other times these would not be present, or if present, in amounts which could not be detected.

The possibility was considered that these might represent conversion products of one of the known steroids. Consequently, hydrocortisone was incubated with adrenal slices. The incubation was carried out with 2.2 g of adrenal cortical tissue slices. Four and seven tenths mg of hydrocortisone were added to the incubating medium which was saline-phosphate (4:1), pH 7.3. Extracts made from the medium and slices were purified by silica gel chromatography. The purified fractions were then chromatographed on filter paper using the technic of Bush(1). In this particular incubation, adrenal slices incubated without added hydrocortisone produced no detectable quantities of the polar compounds, whereas the extracts from the incubation with added hydrocortisone showed the 3 polar compounds on the paper chromatograms (Fig. 1). The hydrocortisone had previously been shown to be chromatographically homogeneous.

The three compounds absorbed ultraviolet light. They reduced alkaline silver diamine and blue tetrazolium dye. They formed orange derivatives of dinitrophenyl hydrazine. The characteristics of these compounds suggest they may be identical with the three compounds "Ai", "Aii", and "Aiii", described by Bush(1) as appearing in the "amorphous" fraction of adrenal extracts.

Incubation of corticosterone with adrenal

* This work was supported in part by contract, DA-49-007-MB-184, Department of the Army, and by Research Grant G-3247 from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Service.