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15962

A Comparison of Effect of Caronamide and Benzoic Acid on Penicillin Plasma Concentrations.

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The excretion of penicillin by the renal tubules can be inhibited by diodrast,[1,2,3] para-aminohippuric acid,[3,4,5] benzoic acid and sodium benzoate,[6,7,8] and caronamide.[9,10,11] Although both diodrast and para-aminohippuric acid have been used clinically to increase penicillin plasma concentrations, the necessity of administering intravenously large quantities of the drugs has limited their general usefulness. Benzoic acid, sodium benzoate and caronamide can be administered orally and may therefore be more widely used in conjunction with penicillin therapy. Since no comparison between the effects of benzoic acid and the new compound, caronamide, has appeared in the literature, such a comparison is here reported.

Plan of Study. Seven individuals who were afebrile and without evidence of cardiac, renal, or hepatic dysfunction, were selected for this study. Each patient was studied during 3 periods and served as his own control. Hospital routine was not modified and no effort was made to limit the intake of food or fluid.

In order that assayable plasma concentrations of penicillin might be anticipated during the entire 3 hour period over which dose response curves were obtained, intramuscular doses of 200,000 units were employed. This dose was dissolved in 2 cc of sterile saline and the sodium salt of penicillin G‡ was used exclusively. The penicillin dose given immediately before the determination of peni-

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"Staticin" caronamide (4'-carboxyphenylmethanesulfonanilide) used in this investigation was supplied through the courtesy of Sharp and Dohme, Inc.

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‡ Commercial Solvents Corporation, Lot No. 46080801, expiration date October, 1949, potency 1572 units/mg.

cillin dose response curves was injected into the right deltoid region by one of the authors and massage of the injection site was avoided.

The pattern of patient study was as follows: *Control day.* An intramuscular injection of 200,000 units of penicillin at 6 and 9 A. M. Following the 9 o'clock injection into the right deltoid region, blood specimens for penicillin assay were obtained at intervals of 15 minutes, 30 minutes, 1 hour, 2 hours and 3 hours (this schedule of sampling is referred to as a dose response curve). *Benzoic acid treatment day.* Each patient was given benzoic acid every 3 hours for 24 hours before a dose response curve was determined. At the same time that the 6 and 9 A. M. doses of benzoic acid were given, an intramuscular injection of 200,000 units of penicillin was administered. Following the 9 o'clock penicillin and benzoic acid medication a dose response curve was obtained. Benzoic acid was administered in 0.5 g gelatin capsules; patients JJ, PR, JC and JA received 2 g every three hours. Patients SB, JG and MV received 3 g every 3 hours. *Caronamide treatment day.* This was similar to the benzoic acid treatment day except that caronamide in 0.5 g tablets was administered in place of benzoic acid.

It should be pointed out that, (a) in all instances at least a three day interval intervened between the benzoic acid and caronamide treatment days; (b) in the case of patients JJ, PR, JC and JA the administration of benzoic acid preceded that of caronamide, whereas patients SB, JG and MV received caronamide before benzoic acid; and (c) patients JJ and SB were incompletely studied so that a caronamide treatment day is lacking for JJ and a benzoic acid treatment day is lacking for SB.

Methods. Blood specimens for penicillin assay were drawn into sterile syringes containing 1 cc of 0.4% sodium citrate solution and were promptly transferred to graduated 15 cc centrifuge tubes. The specimens were centrifuged for 15 minutes at 1500 rpm and the total volume as well as the packed cell volume were recorded. The supernatant plasma was harvested and refrigerated in contact with ice and remained so until penicillin assay on the same or subsequent day. Assays were done by the modified [12] serial dilution method of Rammelkamp using a Group A hemolytic streptococcus as the test organism. All penicillin values recorded in Fig. 1 are corrected for the dilution factor introduced by use of the citrate anticoagulant.

Discussion. It is apparent from the data shown in Fig. 1 that caronamide had a striking effect upon penicillin plasma concentrations when administered orally in doses of 2 or 3 g every 3 hours. Equally apparent is the lack of any significant effect of benzoic acid when given in doses of 2 or 3 g every 3 hours. Six patients showed a marked increase of penicillin plasma concentrations in response to caronamide therapy and 6 patients failed to show any favorable response to benzoic acid treatment. The individual responses to caronamide and to benzoic acid were quite uniform but the averaged data are most striking (Fig. 1). Whereas the benzoic acid dose response curve corresponds exactly to that obtained during the control period, the dose response curve modified by caronamide shows fold increases: at 15 minutes—1.67, 30 minutes—3.57, 1 hr.—3.83, 2 hr.—5.35 and 3 hr.—11.68 (average 5.22) times the control values. The enhancement effects noted in this study are in good agreement with those previously noted. [10, 11]

That benzoic acid had little effect on penicillin plasma concentrations if patients were permitted to have an unrestricted diet was noted by Bronfenbrenner and Favour.[7] These authors sharply restricted fluid and salt intake so that "the urine volume commonly fell to 400 to 600 cc in 24 hours," and gave 2.5 g of benzoic acid in capsules every 4 hours. On this regimen, enhancement effects were demonstrated and were ascribed to benzoic acid.

The patients studied in our series were given food and fluid ad libitum and doses of 16 and 24 g of benzoic acid per day. No elevation of penicillin plasma concentrations

[12] Beyer, K. H., Peters, L., Woodward, Roland, and Verwey, W. F., *J. Pharm. and Exp. Therap.*, 1944, **82**, 312.

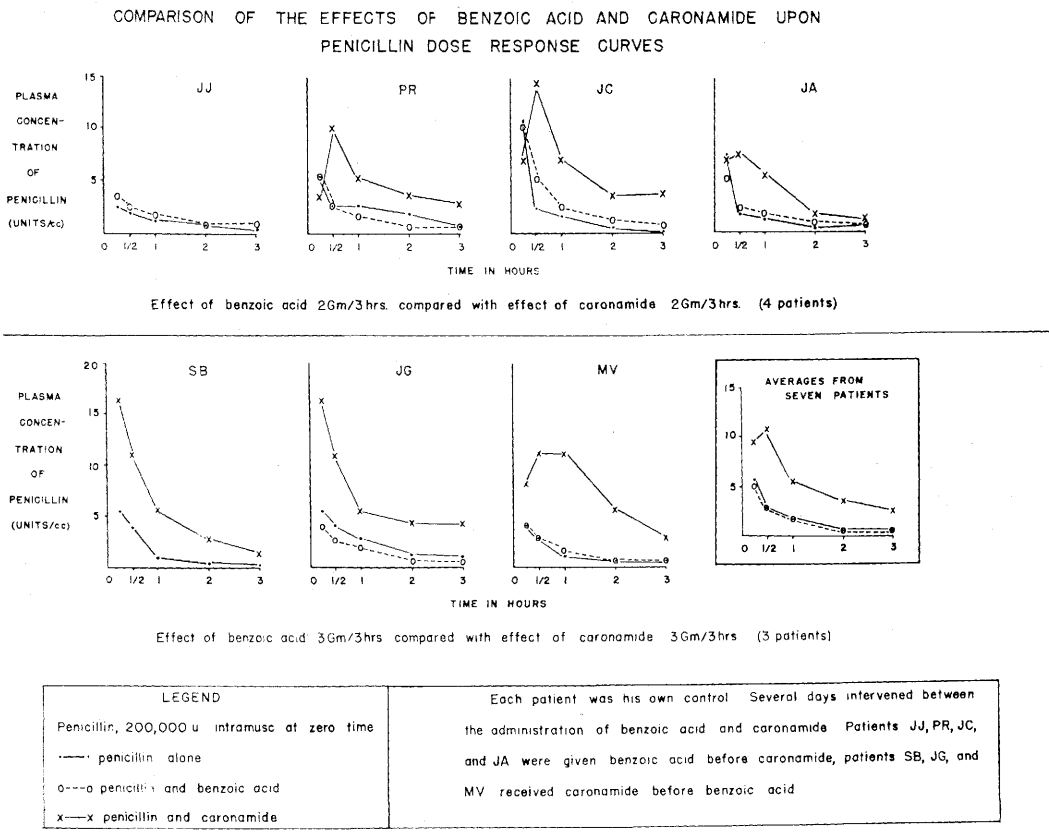

FIG. 1.

was observed. It was suggested that the results reported by Bronfenbrenner and Favour are largely attributable to fluid and salt restriction. The desirability of lowering the daily urinary output to as little as 400 to 600 cc is open to question, especially if patients are suffering from febrile illness. If, however, there is no contraindication to fluid restriction, it can be anticipated that the enhancing effect of dehydration could be superimposed upon the effect of caronamide herein reported.

Spaulding, Bondi and Early[6] reported favorably upon the effect of sodium benzoate in elevating plasma concentrations following the oral administration of penicillin, unrestricted fluid and food intake, and a small dose of sodium benzoate. Bronfenbrenner and Favour indicated that, "on the normal diet, 2.5 g of benzoic acid equalled 6 g of sodium benzoate in raising serum penicillin level." Thus, on an unrestricted diet, Spaulding, Bondi and Early observed a positive effect of a small dose of sodium benzoate, whereas Bronfenbrenner and Favour using a larger dose of benzoic acid, which they regard as a more effective drug than sodium benzoate, observed only a slight effect.

The prolonged enhancing effect of sodium benzoate reported by Bohls, Cook and Potter[8] has been questioned by Spaulding, Bondi and Early on the basis of certain shortcomings of the penicillin assay methods used.

It is quite clear that the few data available on the effects of benzoic acid and sodium benzoate do not permit final evaluation of these two agents as adjuncts to penicillin therapy. If, as indicated by Bronfenbrenner and Favour, restriction of fluid is required to demonstrate an enhancing effect of benzoic acid, use of this agent would have certain disadvantages in the treatment of febrile diseases.

Hippuric acid is formed both in the liver and kidneys by the conjugation of benzoic acid and glycine. Although it is postulated that the hippuric acid thus formed acts in a manner similar to para-aminohippuric acid, it is extremely doubtful whether the amount of circulating hippuric acid following benzoic acid administration is sufficient to inhibit by "mass action" the tubular excretion of penicillin. It is conceivable that benzoic acid may be conjugated in the kidneys and give rise to a "local" high hippuric acid concentration, but this is not subject to proof at present.

In the light of the data here reported, it is our opinion that benzoic acid has no effect upon penicillin plasma concentrations when given in the amounts described.

Conclusions. Benzoic acid given in doses of 16 and 24 g per day to patients on unrestricted diets failed to increase penicillin plasma concentrations. Caronamide given to the same patients in the same doses showed a uniform and marked enhancing effect, averaging five times the control values.

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15963

Further Studies of Effect of Sulphur Compounds on Production of Diabetes with Alloxan.

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In previous publications [1, 2] it was reported that the intravenous injection of large doses of cysteine, glutathione, or thioglycolic acid completely protected rats against a diabetogenic dose of alloxan (40 mg/kg). This protection was observed only when the sulfhydryl compound was administered just prior to the alloxan, or within one minute after the alloxan injection. However if a large dose of cysteine was given 3 or more minutes following the alloxan, no significant protection was observed. It was further suggested that these extraneously administered compounds protected sulfhydryl enzymes from inactivation by alloxan. In this connection it is of interest to note that ascorbic acid, a compound which is reported to decrease the sulfhydryl groups in the body [3] potentiates the effect of alloxan.[4] The more effective production of diabetes in the starved rat [5] may also be related to variation in the glutathione and cysteine content of the body.

Since it was suggested [2] that alloxan may produce its diabetogenic effect by inactivating essential sulfhydryl groups of enzymes, it was desired to test other compounds with known sylfhydryl reactivating effects. The inhibiting effect of arsenic and cadmium on sulfhydryl enzymes has been reported by Peters [6] to be completely reversed by dimercaptopropanol, British Anti-Lewisite, (BAL).

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