

INCREASING AND PROLONGING BLOOD PENICILLIN CONCENTRATIONS FOLLOWING INTRAMUSCULAR ADMINISTRATION¹

Two general methods have been employed in maintaining effective blood levels of penicillin following intramuscular administration; those which delay the absorption of a large dose and those which delay excretion of penicillin. Delayed absorption has been accomplished by giving the penicillin in oil,² in beeswax-peanut oil³ mixtures, in gelatin,⁴ by local ice packs⁵ and with a vasoconstrictor such as adrenalin.⁶ Delayed excretion has been effected by the simultaneous administration of diodrast,⁷ para-aminohippuric acid^{8, 9} and has been observed in subjects with renal failure.¹⁰ Penicillin administered under these conditions may result in a blood level two to four times as high as that obtained by intramuscular injection every 2 to 4 hours. Although 0.03–0.06 units per cc of serum obtained by conventional methods have been considered to be therapeutically effective,^{11, 12, 13} this range of concentration does not exceed the sensitivity level of many of the commonly encountered pathogenic micro-organisms. Furthermore, in certain chronic diseases such as subacute bacterial endocarditis and osteomyelitis it is desirable to maintain higher levels over long periods of time. Since large amounts of penicillin and technical difficulties in administration are factors in obtaining high blood levels, a simple means of obtaining increased blood levels on lower dosages should be useful.

Following the observation that para-aminohippuric acid competes with penicillin for excretion in renal tubules^{8, 9} as has been suggested previously for diodrast and related compounds,⁷ the writers have used

benzoic acid administered orally to accomplish the same purpose. Benzoic acid is detoxified in the normal liver by conjugation with glycine to form hippuric acid. Sodium benzoate has had extensive use in the treatment of rheumatic fever when salicylates can not be used. Daily doses of 12 gm during long periods of time and as much as 50 gm during 1 day have been given without untoward effects.¹³ The penicillin blocking power of benzoic acid, however, may be small on an unrestricted diet. For this reason, fluid and salt restrictions have been combined with benzoic acid therapy (Table 1).

TABLE 1

	G.McL. (I)	M.L. (II)	W.G. (I)	J.M. (I)	W.J. (III)	P.DeG. (II)
Penicillin dosage	20,000 u. q 2h	10,000 u. q 4h	20,000 u. q 2h	20,000 u. q 2h	20,000 u. q 3h	20,000 u. q 3h
Penicillin with normal fluids and normal diet						
30 min. level .	0.14	0.14	0.14	0.28	0.28	
60 " " .	0.07	0.07	0.07	0.14	0.07	
90 " " .	0.07	...	0.07	0.07	trace	
120 " " .	trace	...	trace	trace	0.0	
180 " " .	...	...	...	...	0.0	
2 hr. urine excr.	56%	...	...	...	54.4%	
Penicillin with low fluids and low salt diet						
30 min. level .	0.28	...	0.28	0.56	0.28	0.28
60 " " .	0.14	...	0.21	0.28	...	0.11
90 " " .	...	...	...	...	0.14	...
120 " " .	0.07	...	0.11	0.11	...	trace
180 " " .	0.0	...	...	...	trace	...
2 hr. urine excr.	50.4%	...	...	...	48.8%	...
Penicillin with normal fluids and normal diet plus benzoic acid						
30 min. level .	0.28	0.28	0.56	0.56	0.28	...
60 " " .	0.14	0.14	0.28	0.28	...	...
90 " " .	...	...	...	...	0.14	...
120 " " .	0.07	...	0.14	0.14	...	...
180 " " .	trace	...	...	...	0.0	...
2 hr. urine excr.	46%	...	...	...	49.4%	...
Penicillin with low fluids and low salt diet plus benzoic acid						
30 min. level .	1.12	...	1.12	1.12	0.56	0.56
60 " " .	0.56	...	0.56	0.56	0.28	0.28
90 " " .	...	...	...	...	0.11	...
120 " " .	0.28	...	0.28	0.28	...	0.07
180 " " .	0.11	...	...	...	0.0	...
2 hr. urine excr.	22.4%	...	...	...	46%	...

(I) = Subacute bacterial endocarditis.
(II) = Gonococcal arthritis.
(III) = Syphilis.

Methods: Benzoic acid crystals were given as a suspension in a sweet gelatin dessert or in acacia flavored with syrup of orange to prevent local irritation of the throat during ingestion, or in 0.6 gm gelatin capsules. The usual dose was 2.5 gm or 4 capsules every 4 hours. Most patients preferred the capsules. Sodium penicillin 20,000 units in 2 cc of physiological saline was given intramuscularly every 2 or 3 hours. Penicillin levels were determined on the serum of freshly drawn blood which was allowed to clot before centrifugation. The assays were done by the broth dilution tube method of titration using a standard culture of *Staphylococcus aureus*. Control levels following single intramuscular injections of penicillin in saline agreed closely with those for the same doses reported

¹ From the Medical Service of the Peter Bent Brigham Hospital and the Department of Medicine, Harvard Medical School, Boston.

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⁴ W. M. Parkins, M. Wiley, J. Chandy and H. A. Zintel, *SCIENCE*, 101: 203, 1945.

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⁶ R. T. Fisk, A. G. Foord and G. Alles, *SCIENCE*, 101: 124, 1945.

⁷ C. H. Rammelkamp and S. E. Bradley, *Proc. Soc. Exp. Biol. and Med.*, 53: 30, 1943.

⁸ K. H. Beyer, R. Woodward, L. Peters, W. F. Verwey and P. A. Mattis, *SCIENCE*, 100: 107, 1944.

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¹⁰ C. H. Rammelkamp and C. S. Keefer, *Jour. Clin. Invest.*, 22: 425, 1943.

¹¹ W. E. Herrell, D. R. Nichols and D. H. Heilman, *Jour. Am. Med. Assn.*, 125: 1003, 1944.

¹² C. S. Keefer, R. Dubos, J. S. Lockwood and E. K. Marshall, *Clinics*, 2: 1077, 1944; and C. S. Keefer, W. T. Brown and C. Lyons, *Clinics*, 2: 1094, 1944.

¹³ A. J. Quick, *Jour. Am. Med. Assn.*, 124: 1219, 1944.

by other investigators.^{10, 11, 12, 14} During the control period the patients were placed on a daily intake of 1,500 to 2,000 calories with the diet containing 6 to 10 gm of salt and a fluid intake of 2,500 to 3,000 cc. Penicillin levels were determined after the third day of this regimen. During combined treatment the penicillin was given 20 to 30 minutes after the benzoic acid. At first sodium benzoate was tried, but it was found that the added base neutralized the effects of a controlled salt intake, and in addition the dose of sodium benzoate proved to be somewhat nauseating. On the normal diet 2.5 gm of benzoic acid equalled 6 gm of sodium benzoate in raising the serum penicillin level. During diet restrictions which were in effect 3 days before penicillin levels were determined, the patients received the same caloric intake as previously, but fluids were limited to 1,000 to 1,500 cc a day and salt to 3 gm or less a day. On the restricted fluid intake the urine volume commonly fell to 400 to 600 cc in 24 hours.

Six patients have been studied so far in this series. In addition to determining the degree of elevation and effective maintenance of blood levels, urine levels were done to check the per cent. excretion of penicillin in a 2-hour period following intramuscular injection. The experimental results of this phase of the study are presented in Table 1.

Results: The 1-hour peak serum levels using 20,000 units of penicillin alone were in the 0.07 units per cc range. Two-hour serum levels, however, were ineffective. Using the low salt and fluid diet plus benzoic acid 1-hour levels were 0.56 units per cc and 2-hour levels 0.28 units per cc. A fall from 56 to 22 per cent. in the 2-hour urine excretion of penicillin as a result of diet and benzoic acid treatment reflects a similar trend (Chart 1). This represents an eight-fold increase in, as well as a prolongation of, the penicillin blood level. In the work with adrenalin⁶ using two and one-half times the dose of penicillin, the 2-hour levels were one fourth this amount, except for one case which showed the same level. With repeated injections every 2 to 3 hours, restriction of salt and water intake and administration of benzoic acid every 4 hours, the minimum levels obtained were 0.14–0.28 units per cc of serum. This

is a five to ten-fold increase over the levels usually maintained when two to five times greater doses of penicillin are used. This method of treatment is being applied to penicillin therapy in acute, subacute and chronic conditions, and will be discussed elsewhere.

Summary: (1) Restriction of fluid intake to 1,500 cc and the salt intake to 3 gm a day doubles the penicillin blood level following interrupted intramuscular

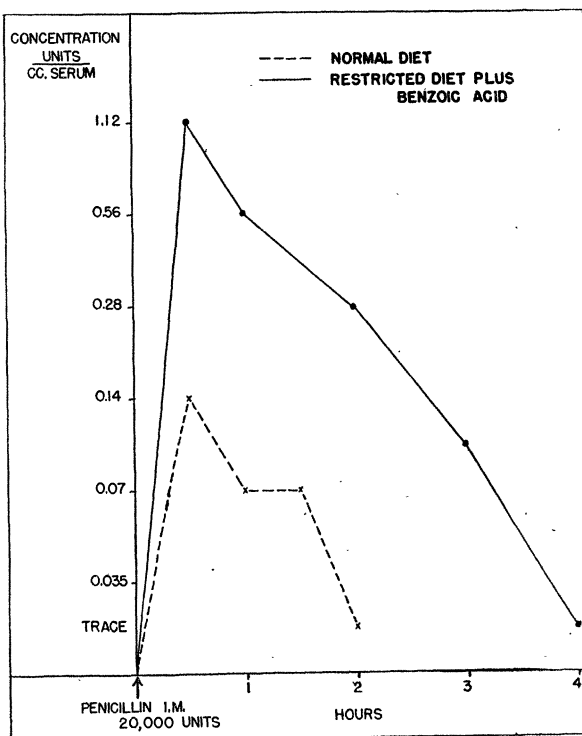


CHART 1

injections of penicillin. (2) The administration of benzoic acid to a patient on an unrestricted diet may double the penicillin blood level during similar treatment. (3) The combination of these two procedures results in a four- to eight-fold increase in penicillin blood level with a prolonged effective blood concentration.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE MICROBIOLOGICAL DETERMINATION OF CHOLINE¹

HOROWITZ and Beadle² have reported a microbiological method for the determination of choline, based

¹⁴ Sir A. Fleming, M. Y. Young, J. Suchet and A. J. E. Rowe, *Lancet*, 2: 621, 1944.

¹ Received for publication, April 11, 1945.

² N. H. Horowitz and G. W. Beadle, *Jour. Biol. Chem.*, 150: 325, 1943.

upon the growth response of *cholinelless*, a mutant of *Neurospora crassa*. The procedure calls for pressing out on filter paper the mold mat obtained at the end of the incubation period, and drying the material to constant weight. The dry weights are reported to vary from 2 to 50 mg for choline concentrations of 0.5 to 20 mcg in 25 ml of media.

In our hands the above procedure gave erratic re-