plication of mumps and influenza (PR8) viruses in tissue cultures at concentrations shown to be without observable toxic effects on the tissue itself. Other analogs tested, including pyridine-3-sulfonic acid, salicyl-β-alanide, 2-amino-4-hydroxy-6-formylpterine, 2,6-diaminopurine, 8-azaguanine, and β-2-thienylalanine, demonstrated no significant inhibitory effects on the growth of these viruses under the conditions of these experiments.

1. Thompson, R. L., *J. Immunol.*, 1947, v55, 345.
2. Morgan, H. R., Abstracts of Papers, 49th General Meeting, Soc. Am. Bacteriol., Cincinnati, May 1949, p82.
3. Morgan, H. R., *J. Exp. Med.*, in press.
4. Eaton, M. D., Magasanik, B., Perry, M. E., and Karibian, D., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 505.
5. Evans, V. J., and Earle, W. R., *J. Nat. Cancer Inst.*, 1947, v8, 103.
6. Hanks, J. H., and Wallace, R. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 196.
7. Ackermann, W. W., *J. Biol. Chem.*, 1951, v189, 421.
8. Umbreit, W. W., and Waddell, J. W., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 293.

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Effect of "Benemid" and Carinamide on Penicillin Therapy of Experimental Pneumococcic Infections in Mice. (19424)

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Carinamide (4'-carboxyphenylmethanesulfonanilide) is a compound capable of inhibiting the excretion of penicillin by the renal tubules(1). Therefore, higher and more prolonged plasma concentrations of penicillin are produced when carinamide is administered during penicillin therapy(2). The oral administration of carinamide was found to enhance the therapeutic effectiveness of penicillin administered intramuscularly 6 to 16-fold in the treatment of experimental pneumococcic infections in mice(3). The enhanced chemotherapeutic effect demonstrated in these experiments has been confirmed clinically(4), but relatively large doses of carinamide were required in order to maintain the blood level of this compound necessary to suppress the tubular excretion of penicillin.

In the continuing search for compounds capable of suppressing more efficiently the tubular excretion of penicillin, 'Benemid',† [p(di-n propylsulfamyl) benzoic acid], was found to be effective in dogs, was well absorbed when given by mouth, and was excreted so slowly that its renal clearance could not be estimated(5). These results suggested the comparative evaluation of carinamide and 'Benemid' for the inhibition of penicillin excretion using dogs, and the results have been reported(5). In addition to these strictly pharmacologic studies, it was considered advisable to compare these two compounds quantitatively in terms of their effects upon the actual chemotherapeutic activity of penicillin in the treatment of an experimental infection in mice. In the experiments to be described, carinamide and 'Benemid' have been compared with respect to their penicillin-enhancing effects and with respect to the time during which the two compounds are able to increase the therapeutic effect of penicillin through the suppression of its tubular excretion. In addition, determinations were made of the blood level curves resulting from the selected doses of carinamide and 'Benemid' so that the potentiating actions of these two

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† 'Benemid' is the trademark applied to p-(di-n propylsulfamyl) benzoic acid by Sharp and Dohme. The tentative nonproprietary name of this compound is probenecid.

drugs could be related to their plasma concentrations.

Experimental. Female mice‡ weighing 16-22 g were used. For the chemotherapeutic experiments, mice were infected intraperitoneally with approximately 1000 minimum lethal doses of a Type I pneumococcus contained in 0.5 ml of a diluted 6-hour broth culture. Intramuscular injections of varying concentrations of aqueous sodium penicillin were given in 0.05 ml quantities at the time of infection, designated as zero hour, and again 12 hours after infection. Five or 10 mg of 'Benemid' or carinamide, suspended in 0.5 ml of 0.5% gum tragacanth, were given by stomach tube at zero hour or at 1,2,4,6,8 or 10 hours before each penicillin injection. Infected control mice receiving penicillin but no adjunct therapy were fed 0.5 ml of the tragacanth suspending material at zero hour. Each treatment group consisted of 10 mice and the experiments were terminated 7 days after infection. The dose of penicillin protecting 50% of the mice (PD_{50}) then was calculated for each treatment schedule, using the method of Reed and Muench(6).

Results. It can be seen that the variable in these experiments is the time between the feeding of carinamide or 'Benemid' and the injection of penicillin; therefore, the greater the persistence of the adjuncts in the blood, the greater and more prolonged would be their effect in reducing the penicillin PD_{50}. The results of the experiments have been expressed as the percentage reduction in the dose of penicillin protecting 50% of the animals (PD_{50}) compared to the control values obtained when no adjunct therapy was administered. The data from several separate experiments have been averaged and the effect of the adjunct pre-feeding time on the penicillin PD_{50} is shown in Table I. It will be seen that when either carinamide or 'Benemid' is given orally at the time of penicillin therapy (zero hour), there is a 90% to 97% reduction in the amount of penicillin required to protect 50% of the treated animals. Further experiments have indicated that this magnitude of decrease represents the maximal enhancement of therapy that can be expected from the complete suppression of renal tubular excretion of penicillin. Absorption of the two adjuncts from the intestinal tract apparently is rapid since no increase in effect is noted when the compounds are fed one hour before the injection of penicillin. These figures also demonstrate that the 5 mg dose of carinamide maintained maximal effectiveness for one hour, but that a 5 mg dose of 'Benemid' maintained the same maximal effectiveness for about two hours. Correspondingly, when the 10 mg doses of these compounds are compared it can be seen that carinamide produces maximum potentiation of penicillin action for approximately 2 hours whereas 'Benemid' maintains its full effectiveness for 8-10 hours. Comparisons drawn from the figures in Table I using other levels of effectiveness confirm the conclusion that the ability of 'Benemid' to support the chemotherapeutic action of penicillin is more prolonged than that of carinamide. These data indicate that when equivalent oral doses are given, 'Benemid' has at least two to three times the duration of action of carinamide.

TABLE I. Percentage Decrease of Penicillin PD_{50} Caused by Adjunct Feeding at Various Times Before Penicillin Therapy.

Hr before penicillin therapy	Carinamide		'Benemid'	
	5 mg	10 mg	5 mg	10 mg
0	90	94	95	97
1	87	94	89	98
2	64	93	90	96
4	29	57	73	92
6	11	26	65	93
8	16	37	23	91
10				85

Since the greater activity of 'Benemid' in enhancing the effectiveness of penicillin could be caused either by its longer persistence in the blood stream or by the possession of a greater intrinsic activity, experiments were undertaken to determine the plasma concentrations produced by equivalent single oral doses of carinamide and of 'Benemid'. For this purpose four large groups of mice were employed. In a series of carefully timed experiments each mouse was fed a single dose of either 5 or 10 mg of 'Benemid' or carina-

‡ Obtained from Carworth Farms, New City, N. Y.

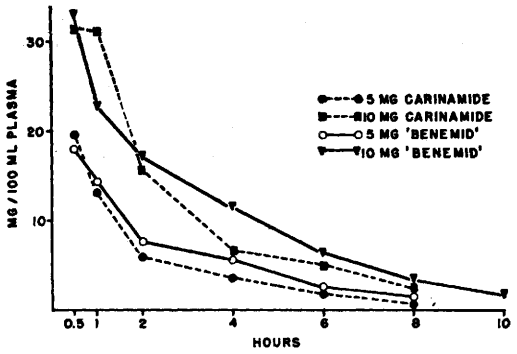

FIG. 1. Average plasma concentrations of carinamide and 'Benemid' in mice produced by single oral doses.

mide. At exactly ½, 1, 2, 4, 6, 8 and 10 hours after the feeding, 17 to 20 mice from each group were exsanguinated under ether anesthesia. Pooled plasma samples were assayed using a modification of the method of Brodie, Levy and Bernstein for the determination of carinamide(7) and both a spectrophotometric and a colorimetric method for 'Benemid'(8).§ The results of several replicated experiments were averaged and are shown in Fig. 1. It will be seen that, although the initial concentrations of equivalent doses of the two drugs are quite similar, the plasma levels of 'Benemid' fall off at a slower rate than do those of carinamide. However, this difference in the rate of disappearance from the blood stream does not appear to be sufficient to explain completely the difference in effectiveness of these compounds in the enhancement of penicillin therapy.

Table II shows the plasma concentration values of carinamide and 'Benemid' that were observed to reduce the PD_{50} of penicillin by 85%, 70%, and 40%. The times at which these percentage reductions occurred were obtained by interpolation on curves constructed from the data of Table I. The plasma concentrations listed in Table II then were read from the blood level curves at the indicated times. It can be seen that carinamide in amounts of 12.5 and 14 mg per 100 ml were required to decrease the penicillin PD_{50} 85%, whereas 'Benemid' at 7 and 1.7 mg per 100 ml accomplished the same reduction. The other figures also demonstrate that equal effects are produced by about one-half as much 'Benemid' as carinamide.

Discussion. The mouse protection data clearly indicate that the effect of 'Benemid' upon the therapeutic activity of penicillin is of considerably longer duration than is the effect of carinamide. The work of Boger, Gallagher, and Pitts on human patients(9) supports this observation.

When the protection test data are considered in conjunction with the information concerning the rate with which these two drugs disappear from the plasma, it becomes apparent that the prolonged effectiveness of 'Benemid' in mice is the result not only of its slower elimination but also of its greater intrinsic activity. Although the renal clearance studies of Beyer, *et al.*, have indicated that equal plasma concentrations of carinamide and 'Benemid' exert approximately equal effects on penicillin excretion in dogs, the data reported here indicate that, in the mouse, 'Benemid' may be at least twice as effective as is carinamide at apparently equivalent plasma concentrations. This seeming discrepancy between the results of the two types of experiment may be explainable on the basis of species differences in the manner in which these two compounds are metabolized. Clinical impressions, based on determination of the plasma concentration of these two drugs found necessary to produce enhancement of the penicillin plasma concentrations in human beings, suggest that the greater effectiveness of 'Benemid' demonstrated in mice also is found in humans(10).

Summary. 1. Oral administration of either carinamide or 'Benemid' enhances the therapeutic effectiveness of intramuscularly in-

TABLE II. Plasma Concentrations of Carinamide and 'Benemid' (mg/100 ml) Required to Produce Specified Reductions of Penicillin PD_{50}.

% reduction of penicillin PD_{50}	Carinamide		'Benemid'	
	5 mg	10 mg	5 mg	10 mg
85	12.5	14	7	1.7
70	8	10	4.5	—
40	4	5.5	2	—

§ These assays were performed through the kind cooperation of Dr. K. H. Beyer and Grace S. Schuchardt of the Pharmacology Section, Research Division, Sharp & Dohme, Inc.

jected penicillin in mice experimentally infected with Type I pneumococci. 2. When equal oral doses of these adjuncts are given, 'Benemid' had at least two to three times the duration of action of carinamide. 3. Plasma concentration studies show that 'Benemid' disappears from the blood stream at a somewhat slower rate than does carinamide. 4. Plasma concentration studies, combined with a consideration of the protection test data, suggest that 'Benemid' has about twice the intrinsic activity of carinamide. 5. The combined data indicate that the prolonged effectiveness of 'Benemid' in mice is the result not only of its slower rate of disappearance from the blood stream but also of its greater intrinsic activity.

1. Beyer, K. H., *Science*, 1947, v105, 94.
2. Crosson, J. W., Boger, W. P., Shaw, C. C., and Miller, A. K., *J. Am. Med. Assn.*, 1947, v134, 1528.
3. Verwey, W. F., and Miller, A. K., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 222.
4. Eisman, S. H., Kay, C. F., Norris, R. F., and Boger, W. P., *Am. J. Med. Sci.*, 1949, v217, 62.
5. Beyer, K. H., Russo, H. F., Tillson, E. K., Miller, A. K., Verwey, W. F., and Gass, S. R., *Am. J. Physiol.*, 1951, v166, 625.
6. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.
7. Brodie, B. B., Levy, B., and Bernstein, E., *J. Pharm. and Exp. Ther.*, 1947, v91, 246.
8. Tillson, E. K., Russo, H. F., Schuchardt, G. S., Gass, S. R., and Beyer, K. H., 1952, to be published.
9. Boger, W. P., Gallagher, M. E., and Pitts, F. W., *J. Phila. Gen. Hosp.*, 1950, v1, 51.
10. Boger, W. P., Personal communication.

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Comparative Study of Anabolic Effects of Three Androgenic Steroids.* (19425)

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It has been reported(1) that testosterone cyclopentylpropionate (hereafter referred to as TCP) has greater anabolic and androgenic activity in rats than testosterone propionate (TP) or methylandrostenediol (MA). Studies in man(2) have shown that TCP has a more effective androgenic action than has TP. As a result of recent clinical experiments(3), it seemed desirable to attempt a comparative evaluation of the anabolic potency in man of these 3 steroidal preparations.

Method. A 56-year-old white man in good nutrition with a gastrostomy for a benign stricture of the esophagus was placed on a constant daily tube diet supplying: protein 70 g (11.2 g nitrogen), carbohydrate 98 g, 1730 calories; sodium 55.5 meq, potassium 70.7 meq, and adequate vitamin supplements all in a volume of 3050 cc. Twenty-four-hour urine specimens were collected and were measured carefully for volume and analyzed for total nitrogen (Koch-McMeekin method), carbohydrate (Nelson-Somogyi method), sodium and potassium (Perkin-Elmer flame photometer). Periodic determinations were made of the 24-hour urinary excretion of 17-ketosteroids (Robbie and Gibson method). Fecal nitrogen was not measured and was assumed to be constant(4). After a 7-day control observation period, and at 7-day intervals, the patient was given an intramuscular injection with equivalent contents of testosterone (2) of each of the 3 steroids in the following order: TP (300 mg), MA (300 mg), and TCP (353 mg).

Results. Fig. 1 presents the metabolic data of the entire experiment graphed according to the method of Reifenstein, Albright, and Wells(5). The apparent nitrogen retention manifested during the control period is accounted for in the calculated but unmeasured fecal losses. The dotted line extending through the experimental periods is the average excretion level of each substance calculated from

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