TABLE I.
Lysozyme Assays of Normal and Pathological Stool Specimens.

Source	Individual lysozyme titers (units per g wet wt)			Mean lysozyme titer
Normal stools	0.5	9.4	4.4	2.7
	0.2	0.8	0.9	
Normal stools (after purging)	0.4	3.9	1.0	1.6
	0.8	1.3	0.1	
	1.6	3.8		
Chronic ulcerative colitis stools	28.3	48.7	22.3	56.0
	49.0	10.5	180.9	
	33.3	47.0	103.7	
	24.2	119.4	4.1	
"Mucus" from chronic ulcerative colitis patients	43.5	80.0	15.7	158.1
	167.0	466.0	176.5	
Ileal stools	0.1	2.0	3.6	2.8
Idiopathic diarrheal stool	5.0			

as 44,400 units in a comparable time period.

The experiments, although limited in number, indicate that lysozyme production is greatly increased in the diseased colon. The low titer of the ileal stools in the disease makes it unlikely that the enzyme is produced outside the colon. The high titer of the "mucus" points to the colonic mucosa as the source of the enzyme, although some microorganisms have been shown to produce small quantities of lysozyme.[2] We propose as a hypothesis that the pathogenesis of chronic ulcerative colitis may be due to a combination of local overproduction of lysozyme followed by a necrotizing action on the mucosa by the indigenous bacterial flora. We assume from analogy with the action of lysozyme on the gastric mucosa that the enzyme prepares the way for the necrotizing effect by a removal of the protective surface mucus. This hypothesis depends on the demonstration of a substrate of lysozyme in the colon.

[2] Meyer, K., Palmer, J. W., Thompson, R., and Khorazo, D., *J. B. C.*, 1936, **113**, 479.

15918

Effect of Caronamide upon Penicillin Therapy of Experimental Pneumococcus and Typhoid Infections in Mice.

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It is now agreed that one of the major disadvantages of penicillin as a therapeutic agent is the rapidity with which the human and animal kidney excretes the drug from the plasma into the urine. Because of this rapid excretion, which occurs both by way of the renal tubules and the glomeruli,[1] relatively large doses must be given every few hours if a detectable plasma concentration is to be maintained. To obtain higher concentrations of penicillin in the plasma, very

[1] Beyer, K. H., Peters, L., Woodward, R., and Verwey, W. F., *J. Pharm. and Exp. Therap.*, 1944, **82**, 310.

much greater quantities are required. The administration of both diodrast[2] and para-aminohippuric acid (PAH)[3,4] simultaneously with penicillin have been used to decrease the excretion of penicillin. Both of these substances, however, must be given in large amounts by continuous intravenous infusion.

This communication reports experiments demonstrating the effect of a chemical compound that has been reported by Beyer[5] to offer a new approach to the problem of decreasing the urinary excretion of penicillin. The compound, 4′-carboxyphenylmethanesulfonanilide, will be reported by Sprague, Ziegler, Miller and Cragoe,[6] and has been called caronamide. It will be referred to by this name throughout this report. Unlike diodrast and PAH, which compete with penicillin on a mass-action basis for the penicillin transport mechanism of the renal tubular epithelium,[1] caronamide does not appear to be excreted by the tubules and is, therefore, believed to suppress penicillin excretion by blocking the specific enzyme system responsible for penicillin transport through the tubular cells. Because tubular elimination is apparently not a factor in the excretion of caronamide it remains in the blood for a longer period of time than does either diodrast or PAH. Therefore, it is possible to use intermittent administration of caronamide to suppress the excretion of penicillin.[7] For this purpose, the oral route of administration is effective since caronamide is rapidly absorbed from the gastro-intestinal tract. A more detailed discussion of the pharmacology, chemistry and mechanism of action of this drug will be published elsewhere.[5-9]

The pharmacological experiments of Beyer *et al.*, using dogs,[7] demonstrated that the intravenous or oral administration of caronamide could suppress and even eliminate the tubular excretion of penicillin when this antibiotic agent was given by either the intravenous or oral route. Although these experiments clearly indicated that greater therapeutic activity could be expected, the actual increase in the *in vivo* bacteriostatic effect produced by such enhanced and prolonged plasma penicillin concentrations obviously could not be determined by this type of experiment. It was, therefore, considered desirable to design experiments in which animals would be infected with lethal doses of microorganisms and treated with graded amounts of penicillin both alone and with caronamide. The survival rates in the groups of animals receiving various doses of penicillin with and without the drug were believed to offer a means of calculating the bacteriostatic dosage-equivalents of penicillin alone and penicillin with the drug. The ratio of these penicillin dosage-equivalents then could be used as an estimate of the *in vivo* bacteriostatic advantage derived from the suppression of penicillin excretion by the administration of caronamide.

Experimental. White Swiss mice weighing 16 to 22 g were used in all tests. An experiment was carried out using a strain of Type I pneumococcus as the infecting organism. Groups containing 20 mice were established, each mouse receiving, by intraperitoneal injection, 0.5 ml of a 1:3,000,000 dilution of a 6-hour broth culture. This amount of culture subsequently was shown, by titration in untreated control mice, to contain approximately 1000 minimum lethal doses of pneumococci. Animals which received combined treatment were given 10 mg of caronamide in 0.5 ml of a 0.5% gum tragacanth suspension by stomach tube and an intramuscular injection of 0.1 ml of 0.85% sodium chloride solution containing 2, 4 or 8 units of penicillin. The animals receiving only penicillin were given a similar intra-

2 Rammelkamp, C. H., and Bradley, S. E., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 30.

3 Beyer, K. H., Flippin, H., Verwey, W. F., and Woodward, W., *J. Am. Med. Assn.*, 1944, **126**, 1007.

4 Loewe, L., Rosenblatt, P., and Alture-Weber, E., *Am. Heart J.*, 1946, **32**, 327.

5 Beyer, K. H., *Science*, 1947, **105**, 94.

6 Sprague, J. M., Ziegler, C., Miller, C. S., and Cragoe, L. J., to be published.

7 Beyer, K. H., Miller, A. K., Russo, H. F., Patch, E. A., and Verwey, W. F., *Am. J. Physiol.*, in press.

8 Crosson, J. W., Boger, W. P., Shaw, C. C., and Miller, A. K., to be published.

9 Beyer, K. H., Rapoport, M., Corneal, F. B., and Verwey, W. F., to be published.

TABLE I.
Survival Percentages of Mice Infected with Type I Pneumococci and Receiving Penicillin with Caronamide and Penicillin Alone.

Penicillin dosage,* units	Penicillin with caronamide†	Penicillin alone
2	0	—
4	35	—
8	84	—
16	—	5
32	—	10
64	—	65
128	—	85
PD_{50}	5.0 units	55 units

* Doses given intramuscularly every 6 hours for 9 doses.
† 10 mg given orally every 6 hours for 9 doses.

muscular injection containing 16, 32, 64 or 128 units of penicillin and an oral dose of 0.5 ml of the gum tragacanth suspending vehicle alone. All the animals, except the culture virulence controls, received this treatment immediately following infection and every 6 hours thereafter for 48 hours (9 doses). Treatment then was discontinued and the mice were observed for 5 additional days. The results of this experiment are given in Table I, which includes also the PD_{50} dose (dose protecting 50% of mice) calculated from the data by the method of Reed and Muench.[10] These latter figures represent the calculated amounts of penicillin that would have saved the lives of 50% of the mice treated by the 2 procedures employed in the experiment outlined above.

The data indicate that penicillin administered intramuscularly was approximately 11 times more effective in the prevention of death from pneumococcal infection when its excretion was decreased by the simultaneous oral administration of caronamide. Two other experiments similar to this, except that 10 mice were used in each group, have indicated equivalent dosage ratios of 6 and slightly greater than 16 in favor of the combined penicillin and caronamide therapy.

Inasmuch as pneumococci are known to be sensitive to very low concentrations of penicillin, experiments of the type described above were carried out using the "Panama 58" strain of *Eberthella typhosa*. Inocula of 10^7 organisms of this strain were found to be almost completely resistant *in vitro* to 2 units of penicillin, partially inhibited by 4 to 12 units and completely inhibited by 14 units per ml of culture medium. Mice were injected intraperitoneally with approximately 10^5 organisms, representing 1000 MLD's, in 0.5 ml of a 5% hog gastric mucin suspension. The groups of infected mice that received by mouth 10 mg of caronamide each were treated with intramuscular injections of 4, 20, 100 and 500 units of penicillin while those receiving no drug were given 20, 100, 500 and 2500 units of this antibiotic agent. As in the pneumococcal experiments, all mice were treated at the time of infection and every 6 hours thereafter for 48 hours. They were then observed for 5 days after the conclusion of the treatment period. The mice that were given penicillin alone received oral doses of gum tragacanth suspension containing no caronamide. Animals that were infected but untreated served as virulence controls.

The results of this experiment indicated that the PD_{50}'s of penicillin, when calculated from the survival percentages, were 320 units for mice receiving caronamide and 1370 units for mice not receiving this drug. The equivalent dosage ratio was, therefore, 4.2. This experiment was repeated using 2-fold increments between successive penicillin doses. The mice receiving 10 mg of caronamide

TABLE II.
Survival Percentages of Mice Infected with *E. typhosa* and Receiving Penicillin with Caronamide and Penicillin Alone.

Penicillin dosage* units	Penicillin with caronamide†	Penicillin alone
100	10.5	—
200	10	—
400	10	10.5
800	95	5.3
1600	—	30
3200	—	90
PD_{50}	515 units	1900 units

* Doses given intramuscularly every 6 hours for 9 doses.
† 10 mg given orally every 6 hours for 9 doses.

[10] Reed, L. J., and Muench, H., *Am. J. Hygiene*, 1938, **27**, 493.

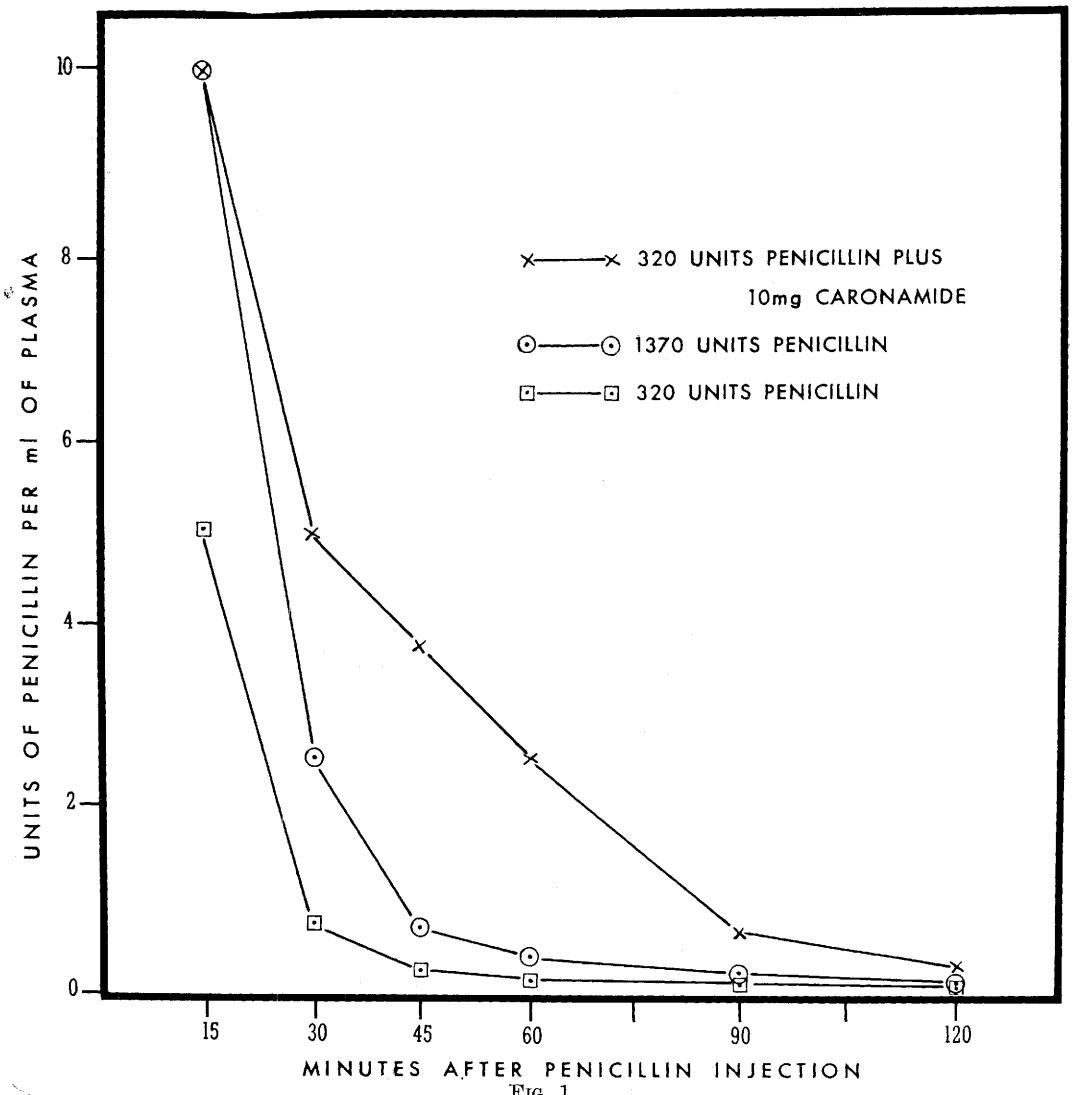

FIG. 1.

Plasma penicillin concentrations in mice following the administration of penicillin alone and penicillin with caronamide.

orally were given 100, 200, 400 and 800 units of penicillin and those receiving no drug were given 400, 800, 1600 and 3200 units. This change in the dosage increments was made for the purpose of increasing the accuracy of the calculations of the equivalent dosage. The results of this experiment are given in detail in Table II. The equivalent doses were 515 units when caronamide was given orally and 1900 without the drug. The ratio of equivalent doses was, therefore, 3.7.

Both the pneumococcus and the *E. typhosa* experiments demonstrated a very considerable increase in the effectiveness of penicillin with caronamide compared to penicillin alone. Experiments were then designed to determine whether doses of penicillin, with and without the drug, that were found to be equivalent in experimental therapy were also equivalent in respect to the height and duration of the plasma concentrations that they produced. Three groups of mice were established: One received 2 doses of 1370 units of penicillin intramuscularly with a 6-hour interval between doses, the second group received 2 doses of 320 units of penicillin in-

tramuscularly according to the same schedule and 10 mg of caronamide orally, and the third group received similar doses of 320 units of penicillin alone. These amounts of penicillin were the equivalent doses calculated from the first experiment in which *E. typhosa* was used. The dosage of 320 units of penicillin without caronamide was included to determine the plasma concentrations produced by this amount of penicillin in the absence of drug. At intervals of 15, 30, 45, 60, 90 and 120 minutes after the second dose, 4, 5 or 6 mice in each group were exsanguinated under ether anesthesia within a 5-minute period and these individual citrated bleedings were pooled. Penicillin plasma concentrations were determined by a modification[1] of the method of Rammelkamp using a hemolytic streptococcus that permitted the detection of 0.019 unit of penicillin per ml of plasma. These data are summarized in the 3 curves of Fig. 1.

It will be seen that the administration of the therapeutically equivalent doses of penicillin with and without caronamide produced plasma penicillin concentrations of quite similar height and duration within the range to which this strain of *E. typhosa* was found to be sensitive in *in vitro* tests. However, as the plasma penicillin concentrations fell to lower levels, there was a marked decrease in the rate of disappearance of penicillin from the blood in animals receiving caronamide. This is illustrated in Table III where the periods of time are given during which the 3 dosage schemes maintained plasma penicillin concentrations at or above various levels. These data give an excellent illustration of the effect of caronamide on the height and duration of penicillin plasma concentrations.

TABLE III.
Duration of Plasma Penicillin Concentrations in Mice.*

Plasma penicillin concentration at or above units	Dosage: Penicillin alone 320 units min	Penicillin alone 1370 units min	Penicillin 320 units Caronamide 10 mg min
8	—	19	21
6	—	23	27
4	19	26	42
2	25	31	68
1	29	41	84
0.5	34	50	101
0.25	43	69	120

* Calculated from Fig. 1.

Discussion. The experiments that have been carried out clearly demonstrate that bacteriostatic action *in vivo* may be achieved with much smaller doses of penicillin when this treatment is combined with the oral administration of caronamide. In addition, it has been shown that the administration of caronamide causes penicillin to attain higher levels in mouse plasma and to remain there for a longer time than when a similar amount of penicillin is administered alone. Other work has indicated that caronamide produces this effect by causing the suppression of the tubular excretion of penicillin.[7] Since the drug has no demonstrable bacteriostatic action of its own and does not cause any enhancement of the *in vitro* bacteriostatic action of penicillin, it cannot be considered to act synergistically with this antibiotic agent. It is believed, therefore, that the increased effectiveness of intramuscularly-administered penicillin results from the influence of caronamide on the duration and magnitude of plasma and tissue concentrations of penicillin.

The strains of Type I pneumococcus and *E. typhosa* used in these experiments were selected as test organisms both because they produced satisfactory experimental infections in mice and because they were organisms having widely different sensitivities to penicillin. It is interesting to note that experiments with these 2 organisms resulted in different equivalent dosage ratios. These were 4 compared to 6-16 for the typhoid and pneumococcus experiments, respectively. The plasma penicillin concentration curves, when considered with the relative penicillin sensitivities of the 2 cultures, offer an explanation for this observation. The intensity of penicillin therapy can be tentatively represented as a function of the area under a curve where the concentration of penicillin is plotted against time and the baseline is the minimal penicillin concentration having any bacteriostatic effect upon the test or-

ganism. In the case of the *E. typhosa* strain, this baseline was approximately 2 units and with the pneumococcus it was 0.0008 unit. It can be seen that as the baseline is moved downward the area under the penicillin-caronamide curve increases disproportionally to that of the curve produced by the administration of penicillin alone. Thus it will be seen that the advantage produced by caronamide in penicillin therapy, though very considerable against organisms of relatively high resistance, becomes even greater against highly susceptible organisms.

Many bacterial infections that have been considered to be resistant to the usual clinical penicillin dosages have been found to be caused by bacteria sensitive to amounts of penicillin that, in these experiments, inhibited *E. typhosa*. Evans[11] has reported that, of 66 strains of *E. typhosa*, all but one were inhibited completely by 10-25 units of penicillin per ml of culture medium. While it is not possible to translate penicillin dosage in mice into human dosage, it seems reasonable to expect therapeutic effects in man at plasma penicillin concentrations similar to those found to be effective in mice. The studies carried out by Crosson, Boger, Shaw and Miller[8] have indicated that such plasma penicillin concentrations may be attained in man with moderate intramuscular doses of penicillin when caronamide is administered orally. It would, therefore, seem that the use of caronamide together with penicillin may offer a means of increasing the effectiveness of penicillin treatment and bringing more bacterial infections within the limits of practical penicillin therapy.

Summary. The oral administration of 4′-carboxyphenylmethane-sulfonanilide (caronamide) was found to enhance considerably the therapeutic effectiveness of intramuscularly-administered penicillin in mice experimentally infected with either Type I pneumococci or *E. typhosa*. This enhancement was 6- to 16-fold in the case of the pneumococcus experiments and approximately 4-fold for *E. typhosa*. These effects may be explained on the basis of the higher and more prolonged plasma penicillin concentrations when caronamide is administered, and do not indicate synergy between caronamide and penicillin. The significance of these observations in relation to penicillin therapy is discussed.

[11] Evans, R. W., *Lancet*, 1946, **2**, 113.

15919 P

Low Sodium-Forced Fluid Management of Hypertensive Vascular Disease and Hypertensive Heart Disease.*

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One hundred patients with essential hypertension were placed for periods of several weeks to 12 months on a diet of 2200 calories containing approximately 200 mg of sodium, 2.2 g potassium, 70 g protein, 80 to 175 g fat, 130 to 230 g carbohydrate, and vitamin supplements. A daily fluid intake of 3 liters was maintained. All were outpatients. Blood pressure readings were taken with the subject reclining, sitting and standing. They were made by one observer usually at the same time of day. No pretreatment blood pressure was below 170/100. Medical and dietary checks were ordinarily made once or twice a month.

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