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Metabolism of Methyl and Benzyl Esters of Penicillin by Different Species.

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The various esters of penicillin are of potential pharmacological interest because of their fat solubility and because under some conditions they may be more stable than the ordinary salts. Mayer, Hobby, and Chaffee[1] first reported preparation of the methyl, ethyl, butyl, and benzohydryl esters, but the preparations used by them were so impure that interpretation of their data is difficult.

Cavillito *et al.*[2] used a preparation described as pure benzyl ester of penicillin G and concluded from studies carried out chiefly in mice that this preparation offers great promise of clinical usefulness. On the basis of metabolic studies in dogs we had previously abandoned work on the methyl ester of penicillin G[3] because it was not hydrolyzed in sufficient amounts to produce a therapeutic level of free penicillin in plasma or urine. Because of the discrepancies between our results and those of Cavillito *et al.* we undertook a comparative study of the metabolism of the methyl and benzyl esters of pure penicillin G by a variety of species of animals.

Methods. Dogs, mice, rats, monkeys, and rabbits were injected subcutaneously with commercial calcium penicillin, pure methyl ester of penicillin G, and the benzyl ester of pure penicillin G.* Samples of blood were removed by cardiac puncture at appropriate intervals of time and the concentration of free penicillin in the plasma determined by an agar cup test.† In the case of dogs complete urine samples were also collected and analyzed for the presence of penicillin. In dogs, monkeys, and rabbits, the same animals were used repeatedly for the study of the 3 preparations of penicillin. Because of the size of the other species it was necessary to sacrifice individual animals in order to obtain sufficient blood for analysis.

Calcium penicillin was administered as an aqueous solution; the methyl ester as a suspension in 2.5% aqueous starch emulsion unless otherwise stated. Because of the physical properties of the benzyl ester, it was administered as a peanut oil solution. Depending on the species, 3000 or 5000 International units of free or potentially free penicillin per kilogram were administered at each injection.

Results. Fig. 1 through 5 summarize the results obtained in the various species. The numbers in parentheses in the legend represent the number of animals used for determination of each point in the curve. Each point is a mean of the individual assays.

In all species administration of calcium penicillin resulted in significant plasma levels which reached a peak in 15-30 minutes, and fell off to zero in 2 hours. In general the larger the animal the higher was the peak plasma level for the same dosage per kilogram. Thus in the dogs which averaged 14.1 kilos,

1 Mayer, K., Hobby, G. L., and Chaffee, E., *Science*, 1943, **97**, 205.

2 Cavallito, C. J., Kirchner, F. K., Miller, L. C., Bailey, J. H., Klimek, J. W., Warner, W. F., Suter, C. M., and Tainter, M. T., *Science*, 1945, **102**L, 150.

3 Richardson, A. P., Ahlgren, M. W., and Miller, I., unpublished data.

* We are indebted to Dr. Elliott Shaw and Mr. Wm. Lott of the Division of Medicinal Chemistry for the sample of benzyl ester, and Dr. Max Adler and Dr. Oskar Wintersteiner of the Division of Organic Chemistry for the sample of the methyl ester. The methyl ester was a crystalline analytically pure preparation with sharp melting point. The benzyl ester was made from pure sodium penicillin G; it was a glassy, slightly colored solid, analyzing approximately for a benzyl ester of penicillin G.

† The biological tests for potency were carried out in the Division of Microbiology under the direction of Dr. Geoffrey Rake and Dr. Dorothy Hamre.

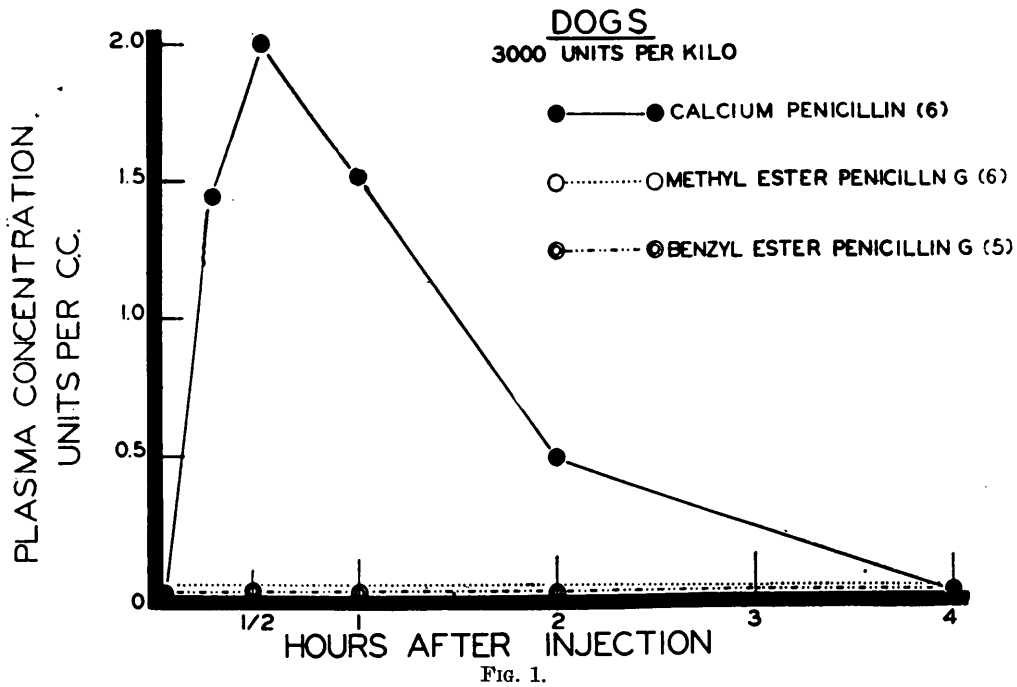

FIG. 1.

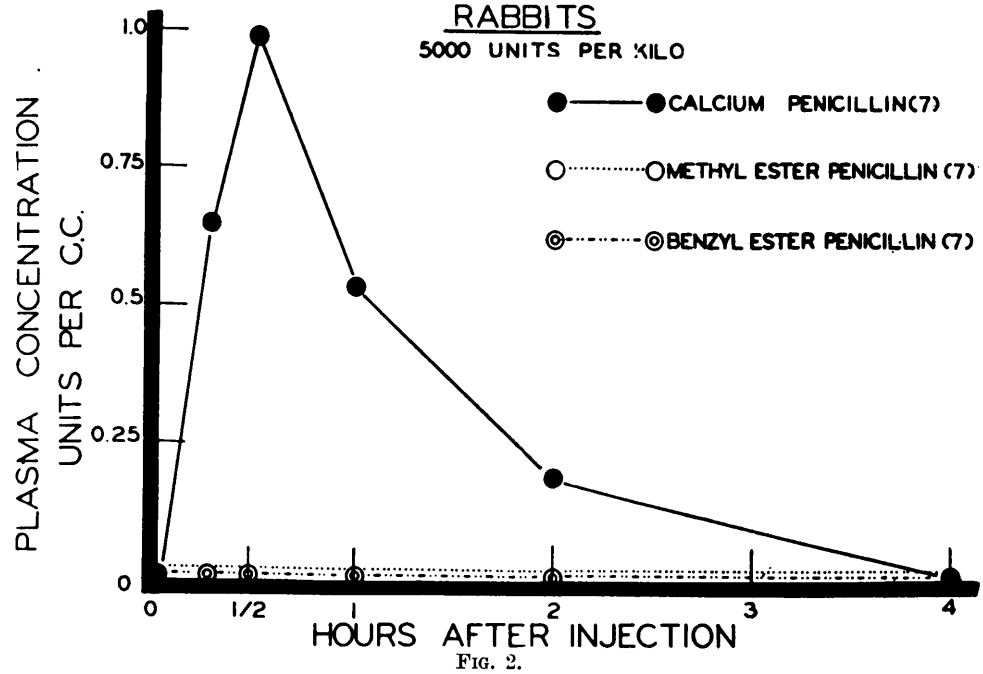

FIG. 2.

3000 units per kilogram gave an average plasma peak of 2.0 units per cc; whereas in mice which averaged 25.0 g, 5000 units per kilogram resulted in a peak level of only 1.1 units per cc.

In contrast to calcium penicillin, the results with the esters showed great differences depending on the species. In monkeys, rabbits, and dogs no free penicillin was detected in the blood at any time following administration.

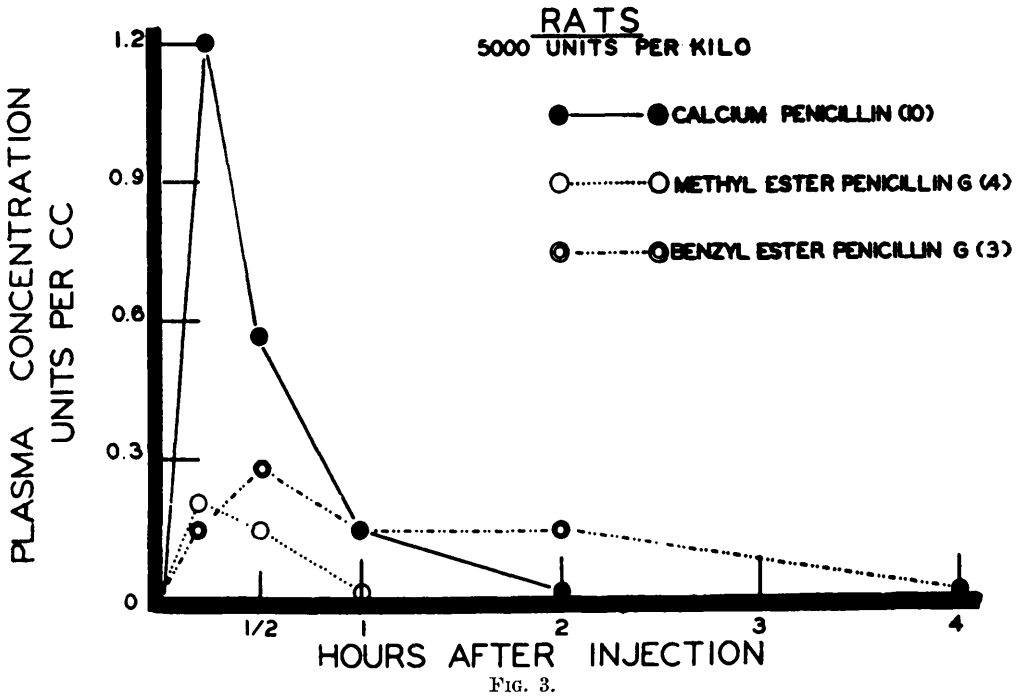

FIG. 3.

In mice, almost equivalent concentrations of free penicillin were observed following administration of the calcium salt and of the methyl ester. Plasma levels obtained with the benzyl ester in this species were somewhat lower, but since it was necessary to administer this preparation in peanut oil, it is likely that the delayed plasma peak noted was due to the vehicle. In confirmation of this conclusion the methyl ester was administered in peanut oil in one set of mice and as can be seen from Fig. 4, lower plasma levels were obtained and the peak of absorption was delayed.

The results obtained with rats were intermediate between those of mice and the other species. With both of the esters, significant levels of free penicillin were observeed in plasma, but not as high as in mice.

The only animals in which urine was collected were dogs. Up to 48 hours after the administration of the benzyl and methyl esters, less than 1% of free penicillin was recovered. This compares with 52% recovery when calcium penicillin was administered.[3]

Limited experiments were carried out in man with the methyl ester. Subcutaneous injection of 50,000 units suspended in starch failed to give a detectable blood level at any time up to 7 hours, and less than 0.5% free penicillin was recovered in the urine.

Discussion. Since neither of the esters used in this study had appreciable *in vitro* activity‡ it is reasonable to believe that any *in vivo* activity they may possess would be dependent on hydrolysis to free penicillin. It is apparent that there is great species difference with regard to hydrolysis of these esters. On the basis of very limited experience our studies indicate that man resembles monkeys, dogs, and rabbits in the manner in which they handle these two penicillin esters and it follows that it is questionable whether they will have any usefulness in man.

We have previously reported on the activity of the methyl ester in the treatment of relapsing fever in mice and found that it had the same activity as pure penicillin G.[4] On

‡ The methyl ester used has an approximate potency of 35 units per milligram, the benzyl ester 10 units per milligram, both determined by the cup test.

4 Richardson, A. P., Walker, H. A., Loeb, P., and Miller, I., *J. Pharmacol.*, in press.

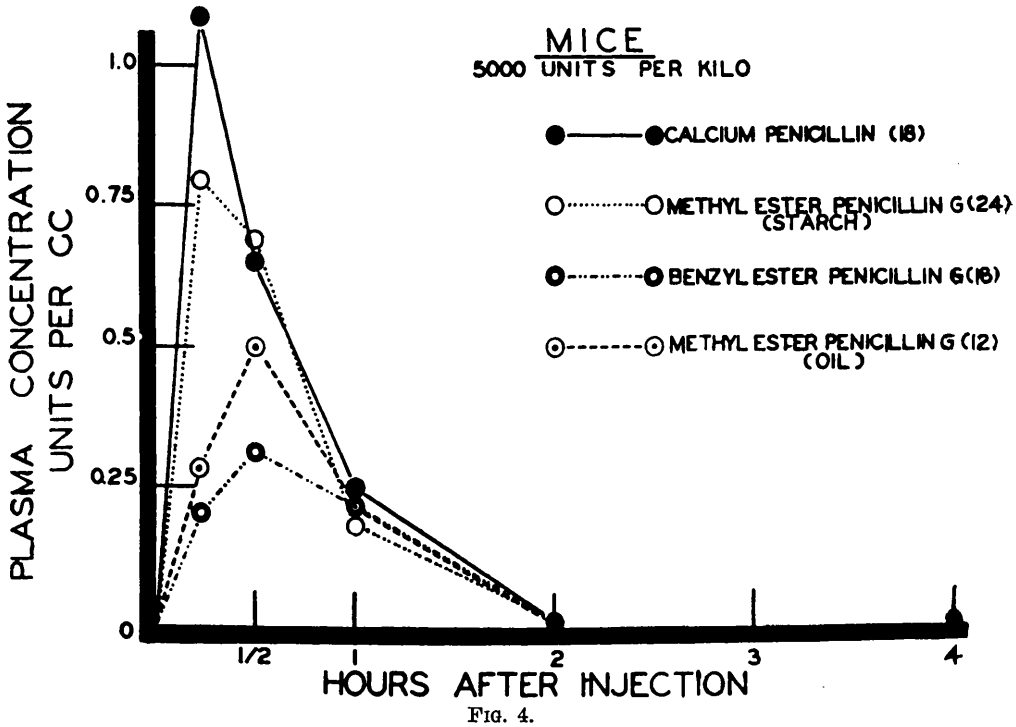

FIG. 4.

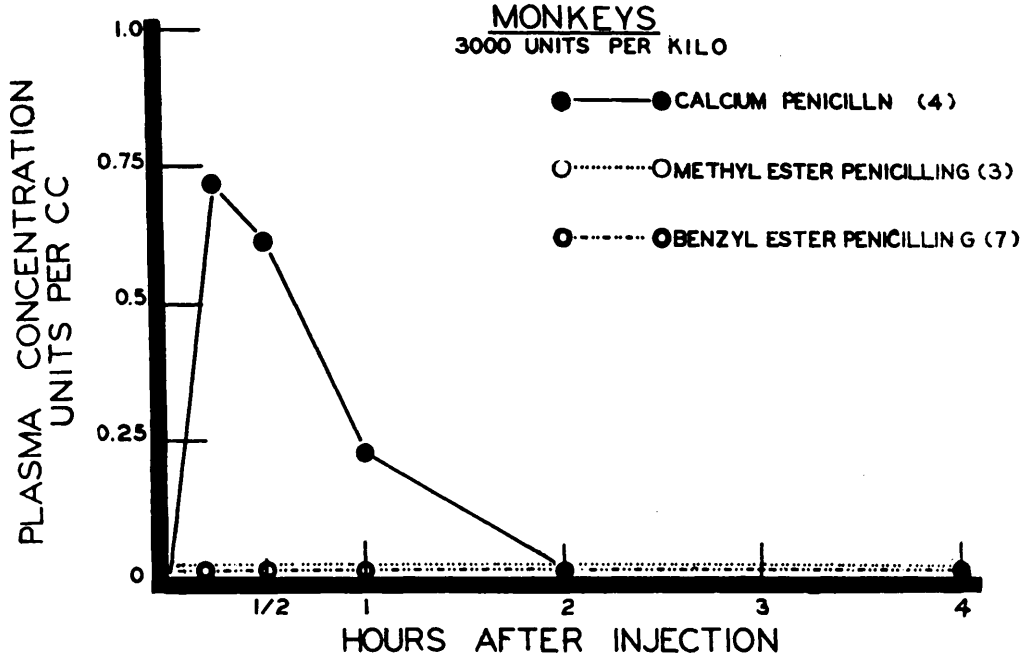

FIG. 5.

Fig. 1-5 represent plasma levels in various species following administration of calcium penicillin, methyl ester of penicillin G, and benzyl ester of penicillin G. Numbers in parentheses are number of animals used for determination of each point. Each point is a mean of all the determinations.

the basis of potentially free penicillin, Cavillito *et al.*[2] reported that the benzyl ester was more active than pure penicillin in the treatment of experimental streptococcal infections in mice. However, the benzyl ester was administered in sesame oil. Since this preparation is oil soluble and water insoluble, it is likely a more prolonged plasma level of free penicillin was obtained with the benzyl ester as compared to the sodium salt. The apparent increased activity of the benzyl ester in their experiments was therefore probably due to delayed absorption, rather than to any inherent increase in activity of the benzyl ester. The evidence available at the present time indicates that it would be hazardous to transfer results obtained in mice to man. Whether man can hydrolyze either of these esters to a significant degree can only be determined by further study.

Conclusions. Plasma levels following administration of benzyl and methyl esters of penicillin G have been determined in a variety of species of animals. Mice hydrolyze both of these esters rapidly and probably completely. Rats are able to hydrolyze the esters less completely. Monkeys, dogs, and rabbits show no appreciable plasma level of free penicillin following subcutaneous injection of ordinary doses of these esters.

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"Renal Hyperlipemia" in Dogs and Rats.*

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Previous work[1] has shown that unilateral and bilateral nephrectomy and also the administration of nephrotoxic agents like mercury bichloride, uranium nitrate, and potassium dichromate regularly increase blood lipid concentration in dogs. Hyperlipemia of the same pattern occurred, however, once after 4 sham operations, three times after 5 splenectomies, and was regularly observed after severe tissue necrosis. It was concluded that the kidneys of dogs, cats, rats, and monkeys are part of a mechanism which influences blood lipid concentration. These studies prompted further investigations to separate more clearly the influence of extrarenal tissue injury from the effect which renal tissue exerts on blood lipid concentration.

Methods and operative procedures have been described previously.[1] In order to eliminate the complicating factor of a general anesthesia, *unilateral nephrectomy* was performed *under local anesthesia* in 3 dogs.† The results are summarized in Table I and are identical with those obtained previously. A temporary hyperlipemia involving cholesterol, phospholipids, and total lipids was observed in all 3 animals. The highest values were obtained 4 to 8 days after operation with return to pre-operative levels after 10 to 14 days. It may be concluded that the general anesthesia used in former studies had no part in the development of the hyperlipemia following renal ablation.

When mercury bichloride was given by intramuscular or subcutaneous injection moderate tissue necrosis, resulting in ulcerations, was occasionally observed. It is conceivable that these lesions had an effect on the temporary hyperlipemia regularly observed after the administration of this nephrotoxic agent. *Mercury bichloride* therefore was *given by intravenous injection* in a 0.5% aqueous solu-

* Aided by a grant from the John and Mary R. Markle Foundation.

1 Heymann, Walter, and Clark, E. C., *Am. J. Dis. Child.*, 1945, **70**, 74.

† We are greatly indebted to Dr. Harry Goldblatt of the Institute of Pathology, School of Medicine, Western Reserve University, for performing the operations on the dogs.