above dogs (Fig. 1). Three of the dogs did not show an increased bromsulphalein retention after the first injection of Mapharsen and subsequent injections also failed to produce demonstrable change. The remaining 5 dogs showed an increased retention of bromsulphalein following the first injection of Mapharsen. However, after the 2 subsequent injections, tolerance had increased in these dogs so that the bromsulphalein retention either approached or returned completely to normal (Fig. 1).

Discussion. Normal dogs are said to tolerate 6 to 8 mg of Mapharsen per kilo without developing liver injury, as judged by the icterus index.[3] Using the bromsulphalein test impairment was detected with doses of Mapharsen as low as 3.4 mg/kg. Thus the bromsulphalein test may be more sensitive than the icterus index in detecting functional hepatic damage in dogs produced by Mapharsen. As can be seen from Table I there is also a marked variation in the liver function response following the injection of Mapharsen, so that in a smaller series of dogs one may easily obtain either a high or low percentage of the dogs showing hepatic damage. When Mapharsen was injected at weekly intervals, dye retention which occurred following the first injection was less following each additional injection. Therefore, one cannot test a given drug against Mapharsen toxicity by injecting Mapharsen one week, and Mapharsen with the substance to be tested the following week. Rats, rabbits and dogs have been reported to acquire a tolerance to certain organic arsenicals,[5] and the present study shows a similar tolerance with respect to the liver function of dogs.

Summary. Fifty-nine per cent of 17 normal dogs developed an abnormal liver function after a single injection of 60 mg of Mapharsen. The dye retention occurred irrespective of dosage over a range of 2.7 to 9.1 mg/kg of body weight. Dogs showing hepatic dysfunction from an initial injection of Mapharsen, subsequently showed less dye retention or a complete return to normal on 2 subsequent injections. Dogs having a normal bromsulphalein retention after one injection of Mapharsen also showed a normal dye retention following two subsequent injections.

[5] Kuhls, M. L., Longley, B. J., and Tatum, A. L., *J. Pharm. and Exp. Therap.*, 1939, **66**, 312.

14931 P

The Prolonging Action of Penicillin by Para-Aminohippuric Acid.*

LEO LOEWE, PHILIP ROSENBLATT, ERNA ALTURE-WERBER, AND MARY KOZAK.
(Introduced by B. Kramer.)

From the Department of Medicine and the Department of Laboratories, Jewish Hospital of Brooklyn.

The successful treatment of certain infectious diseases with penicillin, notably those in which the offending organism is resistant to the drug, certainly is compromised by the wasteful manner in which the human body disposes of penicillin. Beyer, Flippin, Verwey, and Woodward[1] have proposed para-aminohippuric acid as an agent which aids the economy whereby penicillin is utilized by the body. They report that this substance competes with penicillin for the same tubular excretory mechanism and their simultaneous intravenous administration results in augmented blood penicillin levels. In view of the fundamental importance of this observation, it was considered worthy of clinical application in cases of subacute bacterial endocarditis particularly in those patients who were refractory to our standard dosages of penicillin.[2] Limited supplies of para-aminohippuric acid† did not permit extended studies to be made, however, and consequently only preliminary observations are being reported.

Procedure. The subjects received penicil-

* Aided by grants from friends of the Hospital and the Dazian Foundation for Medical Research.

1 Beyer, K. H., Flippin, H., Verwey, W. F., and Woodward, R., *J.A.M.A.*, 1944, **126**, 1007.

TABLE I.
The Enhancement of Penicillin Blood Levels by the Simultaneous Administration of Para-Aminohippuric Acid.

Case No.	Penicillin Oxford units Daily dose (million)	Blood levels	P. A. H. Daily dose (g)	Hour of sample	Blood levels Penicillin Oxf. units	P.A.H. mg %
1 A.S.	0.5	0.375	60.0		1.0	11.44
2 F.L.	0.5	0.5	100.0*	24	2.0	18.79
				6	3.0	18.79
				24	1.5	19.98
3 M.L.	1.0	0.6	100.0*	6	0.43	10.04
				6	0.43	21.60
				24	3.0	21.60
4 S.Z.	2.0	2.4	60.0		4.8	11.88

* 80 g—venoclysis + 20 g—priming.

lin‡ dissolved in a liter of solution by continuous intravenous drip, daily. The vehicle, during control periods, consisted of isotonic saline or Ringer's solution. During the experimental period the diluent consisted of sodium para-aminohippurate in 4-8% concentrations in sterile distilled water. In some experiments "priming doses" of sodium para-aminohippurate were also given twice daily. These consisted of 50 ml of a 20% solution of sodium para-aminohippurate which was injected intravenously. There were, then, 4 phases to the test: an initial control period when only penicillin was administered by continuous venoclysis, a second period when penicillin dissolved with 4-8% sodium para-aminohippurate in water was given, a third phase when priming doses of sodium para-aminohippurate were administered in addition to the maintenance dose of penicillin in sodium para-aminohippurate (second phase), and the fourth period which was similar to the first. Thus there were 2 control periods at the beginning and end of the experiment when only penicillin was administered.

The dosage schedule for penicillin varied from 500,000 to 2,000,000 Oxford units daily. The total daily dosage of sodium para-aminohippurate varied from 40 to 80 g when administered without "priming," plus an additional 20 g when priming dosages were utilized. The maximum amount of para-aminohippuric acid given daily was 100 g, and the patients received this drug continuously for periods up to 8 days. In all instances penicillin assays on serum were performed at varying intervals according to the method of Rosenblatt, Alture-Werber, Kashdan, and Loewe.[3] Para-aminohippuric acid levels were determined by the conventional method described by Goldring and Chassis.[4]

Results. In most instances, it was not possible to achieve high para-aminohippuric acid levels by this method, and consequently no enhancement of the blood penicillin level was apparent. However, in some 50 simultaneous para-aminohippuric acid and penicillin assays there were eight instances in which the blood para-aminohippuric acid level exceeded 10 mg %. It was found (Table I) that at these times the simultaneous penicillin level usually was 3 to 6 times the control figure. In but 2 instances were the penicillin levels below the control.

Discussion. With the technique used, it was difficult to achieve high blood levels of para-aminohippuric acid. Nevertheless, there was usually definite enhancement of the serum penicillin levels when the blood para-aminohippuric acid equalled or exceeded 10 mg %.

The enhancement of serum penicillin levels is obviously dependent upon the maintenance of high blood concentrations of para-aminohip-

[2] Loewe, L., Rosenblatt, P., Greene, H. J., and Russell, M., *J.A.M.A.*, 1944, **124**, 144; Loewe, L., *Bull. N. Y. Acad. Med.*, Feb., 1945; Loewe, L., *J. Canad. Med. Assn.*, 1945, **52**, 1.

† We are indebted to Drs. Karl H. Beyer and John Henderson of Sharp and Dohme, Inc., for the supplies of para-aminohippuric acid used in this study.

‡ We are indebted to Mr. John L. Smith of the Chas. Pfizer Co. for our supplies of penicillin.

[3] Rosenblatt, P., Alture-Werber, E., Kashdan, F., and Loewe, L., *J. Bact.*, 1944, **48**, 599.

[4] Goldring, W., and Chassis, H., *Hypertension and Hypertensive Disease*, Commonwealth Fund, New York, 1944. pp. 203-4.

puric acid. In order to obtain and maintain levels between 30 and 45 mg %, it is necessary to administer the drug at a rate of 150 mg/kg/hr.[1] For an average individual weighing 150 lb (68 kg), this necessitates a daily dosage of 245 g per 24 hr period. Since the daily dosage in our experiments varied between 60 and 100 g of sodium para-aminohippurate, it is not surprising that augmentation of penicillin serum levels was not consistently maintained. On the other hand, such exaggerated serum penicillin levels which were obtained take on added significance when found in conjunction with heightened para-aminohippuric acid levels.

When priming doses of sodium para-aminohippurate were administered, the same smooth muscle effects noted by Beyer and his coworkers[1] ensued. There was a feeling of restlessness and warmth. The face and neck became flushed and there was a sense of cardiac palpitation. The patients noted a burning sensation at the injection site. Several moments later there was a desire for urination and defecation. If the injection was given immediately prior to alimentation there was complete loss of appetite. These symptoms were all mild in character, evanescent, and could be prevented by slowing the rate of injection of sodium para-aminohippurate.

There were no untoward effects noted during the simultaneous administration of sodium para-aminohippurate and penicillin by continuous venoclysis except for an immediate, occasional, slight sense of warmth along the course of the veins which disappeared in a short time. This was more likely to occur with higher concentrations of sodium para-aminohippurate. No cumulative toxic effects were observed after continuous administration of sodium para-aminohippurate for periods up to eight days.

Conclusions. 1. When the plasma para-aminohippuric acid concentration was maintained at or above 10 mg/100 ml a 3 to 6 fold elevation in penicillin plasma concentration was obtained except in two instances. 2. When the para-aminohippuric acid plasma levels were allowed to fall below 10 mg/100 ml there was no effect of that compound on the plasma concentration of penicillin. 3. The para-aminohippuric acid was non-toxic in the dosage given even when administered over a period of 8 days.

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Metabolism of Vitamin D in the Chick.

EVAN W. MCCHESNEY.

From the Research Laboratories of Winthrop Chemical Company, Inc., Rensselaer, N. Y.

In a previous publication dealing with the hypercalcemic effects of activated sterols in the chick[1] some attention was given to the metabolism of vitamin D in this species. It was found that there was excreted in the feces a larger proportion (49%) of an oral dosage of vitamin D_2 than there was of vitamin D_3 (36%). However, in the carcasses of chicks killed 48 hr after the administration of the vitamins, a larger proportion of the vitamin D_3 was found (27% as against 12%). The general purpose of other papers of this series[2,3] has been to obtain information which would serve to explain the remarkably low sensitivity of the chick to vitamin D_2,[4] a characteristic which seems to be shared by turkey poults.[5]

It has been suggested[1] that there appear to be 3 possible causes for the low activity of vitamin D_2 in the chick. One of these, namely, differences in the extent of absorption from the digestive tract, has been dealt with in other papers,[2,3] and it has been shown that such differences can account for no more than half

[1] McChesney, E. W., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 147.

[2] McChesney, E. W., *J. Nutrition*, 1943, **26**, 81.

[3] McChesney, E. W., *ibid.*, 1943, **26**, 487.

[4] Hess, A. F., and Supplee, G. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 609.

[5] Willgeroth, G. B., Halpin, J. L., Halloran, H. R., and Fritz, J. C., *J. Assoc. Off. Agr. Chem.*, 1944, **27**, 289.