

Summary. 1. Marked elevations of serum LDH activity are described in rabbits given *Escherichia coli* endotoxin intravenously. Elevations reached 6 to 50 times the initial values. 2. Alterations in LDH activity of rabbits in endotoxic shock resemble those seen in hemorrhagic shock.

1. Vesell, E. S., Feldman, M. P., Frank, E. D., *Proc. Soc. Exp. Biol. and Med.*, 1959, v101, 644.

2. Fine, J., Frank, H. A., Schweinburg, F. B., Jacob,

S., Gordon, T., *Ann. N. Y. Acad. Sci.*, 1952, v55, 429.

3. Wróblewski, F., LaDue, J. S., *Proc. Soc. Exp. Biol. and Med.*, 1955, v90, 210.

4. Fine, J., Rutenburg, S., Schweinburg, F. B., *J. Exp. Med.*, 1959, v110, 547.

5. Snodgrass, P. J., Wacker, W. E. C., Eppinger, E. C., Vallee, B. L., *N. Engl. J. Med.*, 1959, v261, 1259.

6. Strandjord, P. E., Thomas, K. E., White, L. P., *J. Clin. Invest.*, 1959, v38, 2111.

Received April 19, 1960. P.S.E.B.M., 1960, v104.

6-Aminopenicillanic Acid in Urine after Oral Administration of Penicillins. (25853)

ARTHUR R. ENGLISH, H. T. HUANG AND B. A. SOBIN

Research Labs., Chas. Pfizer and Co., Maywood, N. J., and Groton, Conn.

Sakaguchi and Murao(1) postulated existence of a hydrolytic enzyme capable of cleaving the acyl side-chain from the penicillin molecule. Batchelor *et al.*(2) isolated the actual penicillin moiety, 6-aminopenicillanic acid (6-APA) that results from such cleavage, as a fermentation product of *Penicillium chrysogenum*. Studies to determine whether or not 6-APA could be detected in urine of animal species after oral administration of Penicillin G and several other known penicillins are reported here.

Results. Penicillin G was administered orally at 500 mg/kg to mice housed in metabolism cages. Urine samples (feces free) were collected, pooled, and presence or absence of 6-APA determined by chromatographic procedure described by Batchelor *et al.*(2). The fact that 6-APA *per se* could be absorbed orally and appear in urine was shown by administration of this compound at 50 mg/kg to mice, and its subsequent detection in urine at 2, 6 and 24 hours.

The hydrolytic cleavage of acyl side-chain of Penicillin G *in vivo* was readily indicated by detection of 6-APA in urine of animals after oral administration of the intact antibiotic. 6-APA was detected within ½ hour and at 2, 6, and 24 hours in urine of mouse after oral or intravenous administration of Penicillin G (Table I). Simi-

lar results were found after oral administration of Penicillin G to rats, cats, rabbits, dogs,

TABLE I. Appearance of 6-Aminopenicillanic Acid in Urine of Animals after Administration of Various Penicillins.

Penicillins and dosage in mg/kg	Species	Route of admin.	Presence of 6-aminopenicillanic acid in urine*
6-Aminopenicillanic acid—50	Mouse	PO	Positive
Penicillin			
G—50, 500	Mouse	PO	Positive
V—500	"	"	"
V—"	"	IV	" †
G—"	Rat	PO	"
Dihydro F penicillin—1000	"	"	"
Penicillin			
K—1000	"	"	"
X—"	"	"	"
G—"	Dog	"	"
V—"	"	"	"
G—"	Cat	"	" ‡
G—"	Rabbit	"	"
V—"	"	"	"
G—200,000 units	Man	"	" §
G—500, 1000	Guinea pig	"	Negative
V—"	"	"	"

* Control urines from representative species were always negative. Sample times were at 2, 6, and 24 hr.

† Sample times at ½, 2½, and 3 hr.

‡ " " " 4 hr.

§ 24 hr samples.

TABLE II. Failure of Tissue Macerates to Hydrolyze Penicillins to 6-APA.

Species	Tissue or organ macerate	Conversion of penicillin G to 6-amino-penicillanic acid
Mouse	Kidney	Negative
Rat	Spleen	"
Rabbit	Liver	"
	Lung	"
	Brain	"
Mouse	Intestinal contents	Positive

and man. However, 6-APA was not detected in urine of guinea pig after repeated trials and dosages. Phenoxymethyl penicillin and other known penicillins used instead of Penicillin G gave similar results. (Table I).

Penicillin G was also detected in urines by chromatographic technics after oral administration of this antibiotic. Indications of possible conversion of Penicillin G to parahydroxy penicillin G (Penicillin X) were observed in the chromatographic systems. After oral administration of Penicillin V, presence of parahydroxy penicillin V was indicated by the chromatographic systems. Further experiments are in progress concerning this aspect.

Quantitative conversion in the mouse of administered dose of Penicillin G to 6-APA as appears in urine is quite inefficient, approximately 0.1-1% in these experiments.

In a concomitant series of experiments, enzymatic capacity of various macerated tissues from animal species to hydrolyze Penicillin G to 6-APA was determined. Animals were sacrificed, the individual organs removed, and macerated in aseptic manner in tissue grinder. Penicillin G (200 μ g/ml; 1 mg/ml) was added to macerates and incubated 2-4 hours at 37°C in water bath. Enzymatic conversion of Penicillin G to 6-APA failed to occur with a variety of macerates from different animals under our conditions (Table II). When Penicillin G was added to a water suspension of intestinal tract contents of the mouse, 6-APA was detected. It appears that tissue macerates of the mouse, rabbit, guinea pig, and rat did not contain penicillin acylase activity. It might be postulated that presence of 6-APA in urine results from hydrolytic

conversion of Penicillin G to 6-APA by the microbial flora in the intestine and subsequent absorption and excretion of 6-APA.

Possibly *in vivo* failure of penicillin conversion to 6-APA in the guinea pig is a reflection of an unusual microbial intestinal flora in this species, *i.e.*, *Escherichia coli* is either not found at all in healthy guinea pigs, or found in rare cases and in minimal numbers. The predominant flora of guinea pig intestine are Gram positive, *Bacillus*, *Lactobacillus*, etc. (3,4).

Relevant to appearance of 6-APA in urine of the mouse after intravenous administration of Penicillin G, one might postulate appearance of Penicillin G in the intestine *via* transport in bile, subsequent penicillin acylase activity of intestinal flora, and absorption of 6-APA from intestine and excretion in urine.

Presence of penicillin acylase activity in a number of different microorganisms, such as genera *Escherichia*, *Hemophilus*, *Alcaligenes*, *Micrococcus*, *Pseudomonas*, and *Nocardia* is described by Huang *et al.*(5).

As noted elsewhere, flora of the guinea pig are predominantly *Bacillus* and *Lactobacillus*. These genera failed to convert Penicillin G to 6-APA.

Summary. 1) After oral and intravenous administration of Penicillin G and other known penicillins to mice, and oral administration to rats, dogs, rabbits, and man, 6-APA was detected in urine. After oral administration of penicillin to guinea pigs 6-APA was not detected in urine. 2) Tissue macerates of these animals failed to convert Penicillin G to 6-APA. 3) Probably 6-APA in urine is the result of microbial hydrolytic conversion of penicillin in the intestine and subsequent absorption and excretion.

1. Sakaguchi, K., Murao, S. J., *Agric. Chem. Soc. Japan*, 1950, v22, 411.

2. Batchelor, F. R., Doyle, F. P., Nayler, T. H. C., Rolinson, G. N., *Nature*, 1959, v183, 257.

3. Toshkov, A., Sheikova, G., Shipolini, R., *J. Microbiol., Epidemiol. Immunobiol.*, 1958, v29, 1056.

4. Fischer, G. W., *Zbl. Bakt. Abt. Orig.*, 1955, v164, 1230.

5. Huang, H. T., English, A. R., Seto, T. A., Shull, G. M., Sobin, B. A., *J. Am. Chem. Soc.*, 1960.

Received April 21, 1960. P.S.E.B.M., 1960, v104.