UDC 615.778.25-092.21

150. Toyozo Uno, Hiroyuki Yasuda, and Yutaka Sekine : Studies on the Metabolism of Sulfadiazine. I. On the Separation and Identification of Excrements in Human Urine after its Oral Administration.*1

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Previously, several reports were published on the metabolic fates of such sulfa drugs as sulfisoxazole,[1~4] sufathiazole[5,6] and sulfamethylthiadiazole[7] in our laboratory.

Although sulfadiazine is one of the most widely used sulfa drugs, only a few metabolites have been reported. Namely, Smith and Williams[8] detected its acetyl derivative and Bray, *et al.*[9] detected a hydroxyl derivative as glucuronide in rabbit urine. Recently Ogiya[10] detected an N-glucuronide in rabbit urine after administration of sulfa drugs. However, the metabolic fate of sulfadiazine has not been studied in detail. In the present paper the excrements in human urine after oral administration of sulfadiazine was examined by paper chromatography and paper electorophoresis. As the matabolites, N^4-acetylsulfadiazine, sulfadiazine-N^4-glucuronide, sulfadiazine-N^4-sulfonate and sulfanilamide were found to be present in human urine, but we could not find any other glucuronides and hydroxylated substances.

Experimental

I Preparation of Sample Solution——Human urine was collected for 12 hr. after oral administration of 1 g. of sulfadiazine and filtered through cotton-wool, and was concentrated to a volume of about 1/30 under reduced pressure at temperature below 37°. Salt precipitated was removed with centrifuging after addition of about two times volume of EtOH. The supernatant solution was used for paper chromatography and paper electrophoresis.

II Identification of Each Excrement——Paper chromatography and peper electrophoresis were employed for the identification. Metabolites were compared with synthesized substances using various developing solvents.

a) Paper chromatography

Paper : Toyo Roshi No. 51

Solvent : BuOH saturated with H_2O, BuOH-AcOH-H_2O (5:1:4)
BuOH saturated with 3% NH_4OH, BuOH-PrOH-H_2O (2:1:1)
BuOH-PrOH-0.1*N* NH_4OH (2:1:1), BuOH saturated with *N* HCl

Method : On the Toyo Roshi No. 51A 40×40 cm., sample solution was put on a line and was developed with solvents described above for 15 hr. at room temperature by ascending chromatography. The section of each spot was cut off and eluted with EtOH or H_2O respectively.

b) Paper electrophoresis

Method 1

Paper : Toyo Roshi No. 50 2×40 cm.

Solution : 0.05*M* Na_2HPO_4 solution

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*1 This work was reported at the 16th Annual Meeting of the Pharmaceutical Society of Japan, November 3, 1962.

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1) T. Uno, M. Kono : Yakugaku Zasshi, **80**, 201 (1960).
2) *Idem* : *Ibid.*, **81**, 72 (1961).
3) *Idem* : *Ibid.*, **81**, 192 (1961).
4) *Idem* : *Ibid.*, **81**, 1509 (1961).
5) T. Uno, M. Ueda : *Ibid.*, **80**, 1785 (1960).
6) *Idem* : *Ibid.*, **82**, 759 (1962).
7) T. Uno, U. Okazaki : *Ibid.*, **80**, 1682 (1960).
8) J.N. Smith, R.T. Williams : Biochem. J., **42**, 351 (1948).
9) H.G. Bray, H.J. Lake, W.V. Thorpe : *Ibid.* **48**, 400 (1951).
10) S. Ogiya : Yakugaku Zasshi, **80**, 1538 (1960).

Method : 12~20 v/cm., 4 hr.
Method 2
Except using acetic acid solution at pH 2, other conditions were the same as those in method 1.
c) Detecting reagents
Ehrlich's reagent (for detection of amino group) : 2% *p*-dimethylaminobenzaldehyde EtOH solution containing a volume of 1/50 of conc. HCl.
Aniline hydrogen phthalate reagent (for detection of glucuronic acid) : 1.66 g. of phthalic acid and 0.93 g. of aniline was dissolved in 100 ml. of BuOH saturated with H_2O.
$FeCl_3$ reagent (for detection of phenolic hydroxyl group) : 2% $FeCl_3$ solution.
Gibbs' reagent (for detection of phenolic hydroxyl group) : 0.5% 2,6-dichloro-N-chlorobenzoquinone imine EtOH solution.

III Preparation of Standard Substances——Sulfadiazine : Commercially available sulfadiazine was purified by recrystallization from EtOH, m.p. 254°.
Sulfanilamide : Commercially available sulfanilamide was purified by recrystallization from H_2O, m.p. 165°.
N^4-acetylsulfadiazine : N^4-acetylsulfadiazine was prepared by acetylation[11] in pyridine with excess acetic anhydride and recrystallization from a large amount of EtOH, m.p. 258°.
Sodium sulfadiazine-N^4-glucosiduronate : This compound was prepared by the method of Ogiya and Hashimoto.[12]
Potassium sulfadiazine-N^4-sulfonate : The method of Uno and Kono[1] was applied to the preparation of this compound. 2 g. of $ClSO_3H$ was slowly added to 50 ml. of anhyd. pyridine under stirring and cooling below 15°. To the mixture 5 g. of sulfadiazine was added under stirring and stirring was continued for 2 hr. between 15° and 20°. After the mixture was allowed to stand overnight at room temperature, the mixture was poured into 40 ml. of *N* KOH, and then most pyridine was removed by extraction with Et_2O several times. Water layer was concentrated under reduced pressure and was allowed to stand overnight in a refrigerator. A white precipitate produced was collected and purified by reprecipitation with a small amount of H_2O and EtOH, m.p. > 280°. Yield 1.0 g.

Results and Discussion

The separation and identification of excrements.

The sample solution was examined by paper chromatography used several kinds of solvents and compared with the case of normal urine. Five spots were detected and named as Nos. I, II, III, IV, and V, respectively. The Rf values of excrements are shown in Table I.

Each spot was identified as follows :

Table I. Rf Values of Excrements in Human Urine after Administration of Sulfadiazine

Solvent / Spot No.	BuOH satd. with H_2O	BuOH–AcOH–H_2O (5:1:4)	BuOH–PrOH–H_2O (2:1:1)	BuOH satd. with 3% NH_4OH
I	0.68 (0.68)[a]	0.76 (0.76)	0.74 (0.74)	0.10 (0.10)
II	0.73 (0.73)	0.84 (0.85)	0.82 (0.81)	0.18 (0.18)
III	0.005 (0.005)	decomp. (decomp.)	0.01 (0.01)	0.00 (0.00)
IV	0.045 (0.045)	0.21 (0.20)	0.09 (0.09)	0.00 (0.00)
V	0.50 (0.49)	0.60 (0.60)	0.53 (0.56)	0.49 (0.48)

Solvent / Spot No.	BuOH–PrOH–0.1*N* NH_4OH	BuOH satd. with *N* HCl	Substance
I	0.11 (0.12)	0.72 (0.72)	Sulfadiazine
II	0.18 (0.17)	0.78 (0.78)	N^4-Acetylsulfadiazine
III	0.00 (0.00)	decomp. (decomp.)	Sulfadiazine-N^4-glucuronide
IV	0.01 (0.01)	decomp. (decomp.)	Sulfadiazine-N^4-sulfonate
V	0.55 (0.54)	0.54 (0.54)	Sulfanilamide

a) The values in the parentheses are Rf values of standard substance dissolved in the same sample urine.

11) J.B. Ziegler, A.C. Shabica : J. Am. Chem. Soc., **76**, 594 (1954).
12) S. Ogiya, K. Hashimoto : Yakugaku Zasshi, **81**, 347 (1961).

Spot No. I developed yellow color promptly by spraying the Ehrlich's reagent and the Rf value corresponded with that of sulfadiazine on the each solvent. As sample solution was treated by paper electrophoresis, it travelled to positive. The section of spot No. I which was separated by ascending chromatography using the solvent, butanol saturated with water, was cut off, eluted with ethanol and again developed with other solvents. The Rf value corresponded with that of sulfadiazine. From these facts spot No. I was confirmed as unchanged sulfadiazine.

Spot No. II turned yellow by spraying the Ehrlich's reagent after hydrolysis with 10% hydrochloric acid, and its Rf value corresponded with that of N^4-acetylsulfadiazine. The sample solution was developed on a large filter paper 40×40 using butanol saturated with 3% ammonium hydroxide solvent, and the section of spot No. II was cut off and eluted with ethanol. After hydrolysis with 10% hydrochloric acid in a steam bath for 15 minutes ethanol solution was concentrated to a small volume under reduced pressure and again developed. The Rf value in this case corresponded with that of sulfadiazine. From these facts spot No. II was identified as N^4-acetylsulfadiazine.

Spot I and II were often too close to be separated distinctly, so that method 2 described in the part of experimental method was used, and two spots were separated distinctly.

Spot No. III was detected after about ten second by spraying the Ehrlich's reagent. By aniline hydrogen phthalate reagent this spot turned brown when it was heated at 110°. In the case of paper electrophoresis, it travelled to positive. When acid solvent such as BuOH-AcOH-H_2O or BuOH saturated with *N* HCl was used, the decomposition of this substance to sulfadiazine and glucuronic acid was observed. This property and the Rf value corresponded with those of synthesized sodium sulfadiazine-N^4-glucosiduronate. Spot No. III was confirmed as sulfadiazine-N^4-glucuronide.

Spot No. IV did not develope yellow color promptly by spraying the Ehrlich's reagent but after about ten minutes it developed yellow color. It was decomposed into sulfadiazine and sulfuric acid in the course of development by a solvent of butanol saturated with *N* hydrochloric acid, but not decomposed by several kinds of solvents expect for butanol saturated with *N* hydrochloric acid. This property and the Rf value corresponded with those of synthesized potassium sulfadiazine-N^4-sulfonate. From these facts spot No. IV was identified as sulfadiazine-N^4-sulfonate.

Spot No. V was detected by spraying the Ehrlich's reagent and the Rf value corresponded with that of sulfanilamide, but this metabolite was found only a trace.

For the detection of phenolic hydroxyl compound, Gibbs' reagent or ferric chloride reagent was used after the sample solution was hydrolized with 10% hydrochloric acid for 30 minutes, but none of the positive spot with these reagent was observed other than the spot detected in normal urine. Bray, *et al.* have reported 3-hydroxysulfanilamide and 3-hydroxysulfanilic acid were detected in rabbit urine after sample solution was hydrolized with 5*N* hydrochloric acid[9]. This method was applied for the hydrolysis of the sample solution, and it was compared with synthesized 3-hydroxysulfanilamide[13] and 3-hydroxysulfanilic acid[9] on the paper chromatogram. If these hydroxyl compounds were present in the sample solution, color reaction should be observed by Gibbs' reagent or ferric chloride reagent. However, none of the corresponding spot with these reagent was observed in the case of the sample solution. Accordingly, 3-hydroxyl derivatives are not likely produced in man.

As far as unchanged sulfadiazine, N^4-acetylsulfadiazine and sulfadiazine-N^4-glucuronide were concerned, the authors' result agreed with those of Smith and Williams,[8] and Ogiya.[10] Besides theses substances sulfadiazine-N^4-sulfonate and sulfanilamide were

13) W.V. Thorpe, R.T. Williams : Biochem. J. **35**, 61 (1941).

identified, but its hydroxylated derivative which was reported to be found as glucuronide[9] in rabbit urine was not detected. Other N-glucuronides, namely N^1-glucuronide detected from sufathiazole[14] and $N^{2'}$-glucuronide from sulfisoxazole[15] were not found in the case of sulfadiazine.

Previously sulfanilamide was also detected in the case of sulfisoxazole using same method as reported in this paper. This fact indicates that sulfadiazine which was N^1-heterocyclic derivative of sulfanilamide was also hydrolized to sulfanilamide in human body.

The fact that sulfadiazine-N^4-sulfonate was found showed that N^4-sulfonation took place as in the case of other aromatic amine such as 2-naphthylamine,[16,17] sufisoxazole,[1] sulfathiazole.[5]

The authors express to gratitude to Dr. M. Kono for his helpful advices.

Summary

Metabolic product of sulfadiazine excreted in the urine after its oral administration was examined chiefly through paper chromatography and paper electorophoresis. It was thereby found that the substances excreted are sulfadiazine, N^4-acetylsulfadiazine, sulfadiazine-N^4-glucuronide, sufladiazine-N^4-sulfonate and sulfanilamide.

(Received November 24, 1962)

14) T. Uno, M. Ueda : Yakugaku Zasshi **83**, 200 (1963).
15) T. Uno, M. Kono : *Ibid.*, **82**, 1660 (1962).
16) E. Boyland, D. Manson : Biochem. J., **60**, ii (1955).
17) E. Boyland, D. Manson, S.F. Orr : *Ibid.*, **65**, 417 (1957).