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# Biopharmaceutical Studies of Thiazide Diuretics. III. *In Vivo* Formation of 2-Amino-4-chloro-*m*-benzenedisulfonamide as a Metabolite of Hydrochlorothiazide in a Patient

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Urine, plasma and erythrocytes from a patient receiving hydrochlorothiazide were examined by high-performance liquid chromatography. The urine and erythrocyte showed an unknown peak in the chromatograms. This substance was identified as 2-amino-4-chloro-*m*-benzenedisulfonamide and the identification was confirmed by comparison of the substance with an authentic sample obtained by synthesis. All data were analyzed with a PC 9801VM computer in conjunction with a JASCO MULTI-320 multi-wavelength ultraviolet detector.

**Keywords**——hydrochlorothiazide; hydrolysate; 2-amino-4-chloro-*m*-benzenedisulfonamide; plasma; urine; erythrocyte; nephrotic patient; metabolite

We have investigated the pharmacodynamics of hydrochlorothiazide (HCT), a widely used diuretic, in patients in order to clarify the relationship between the absorption behavior and the clinical responce.[1–4] Beermann *et al.*[5] have reported a bioavailability study with $^{14}$C-HCT in 5 healthy volunteers. They found that over 95% of the absorbed or injected $^{14}$C-HCT was excreted unchanged and that the ethyl acetate extracts of the urine samples collected from almost all volunteers showed only one radioactive spot on the thin-layer chromatogram (TLC). They also found that some labeled material accumulated in an area that did not correspond to HCT in a few samples obtained from only one volunteer, but the amount of the material was less than 0.5% of the excreted radioactivity. There is no other information on the metabolites of HCT. We have sometimes found an unknown peak which seemed to be a metabolite on the high-performance liquid chromatograms of a patient's urine, plasma and erythrocyte. The aim of the present study was to identify the metabolite of HCT.

## Experimental

**Materials**——Powder and tablets (Esidrex®) of HCT were obtained from Ciba-Geigy (Japan) Ltd. 2-Amino-4-chloro-*m*-benzenedisulfonamide (ACBS) was prepared by heating HCT with 10 N NaOH for 1 h at 95 °C.[6] After the solution had cooled, it was acidified by adding HCl. The white precipitate was separated on a glass filter, washed with distilled water, and dried under reduced pressure. The product was isolated by column chromatography on silica gel (22 × 450 mm, Wako Gel C-200, Wako Pure Chemical Ind., Ltd., Osaka) using ethyl acetate–chloroform (30 : 1, v/v).

**Apparatus and Conditions**—The high-performance liquid chromatography (HPLC) system consisted of a TRIROTAR-II pump, a MULTI-320 multi-wavelength ultraviolet (UV) detector (Japan Spectroscopic Co., Japan) and a PC-9801 VM computer (NEC, Japan). The column (250 × 4.6 mm, i.d.) was packed with 5-μm diameter silica gel (Fine SIL-5, Japan Spectroscopic Co., Japan). A precolumn (23 × 3.8 mm, i.d.) of porous silica gel (Perisorb A, Merck) was fitted to protect the main column from plasma or urinary components. The mobile phase was ethanol–dichloroethane–hexane (14 : 10 : 74, v/v) for plasma or erythrocyte and ethanol–hexane (24 : 76, v/v) for urine. The

flow rate was 2 ml/min.

**Determination of ACBS and HCT in Urine, Plasma and Erythrocyte**——The extraction and clean-up procedures were described previously.[2)]

**Clinical Study**——A single dose of 100 mg of HCT (as four tablets of Esidrex®, 25 mg) was administered to a nephrotic patient (female, 56 years old, body weight 55 kg) at 9 a.m. Informed consent was obtained from the patient beforehand. Blood samples (2 ml) were taken from the forearm vein into a heparinized syringe, and centrifuged immediately to separate the plasma and erythrocyte. Urine was collected up to 24 h postdosing. All samples were stored frozen at −20 °C until assayed.

## Results and Discussion

The chromatograms obtained from the patient's plasma, erythrocyte and urine are shown in Fig. 1. These chromatograms were monitored by measuring the absorbance at 270 nm. The extracts from the urine or plasma sample showed a small unknown peak, which seemed to be a metabolite, just behind hydroflumethiazide as an internal standard. In the chromatogram obtained from the erythrocyte sample the unknown peak was much higher than in the cases of the other samples and showed a peak height comparable to that of HCT. The three-dimensional chromatogram of the extract from the erythrocyte sample is shown in

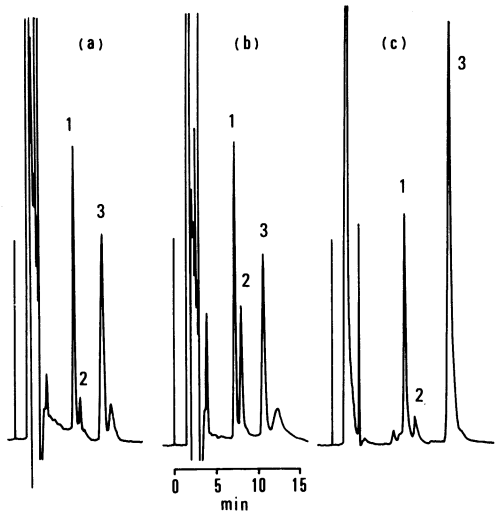


Fig. 1. Typical Chromatograms Obtained from Plasma (a), Erythrocyte (b) and Urine (c) Samples of a Patient

1, hydroflumethiazide; 2, unknown; 3, hydrochlorothiazide. The plasma and erythrocyte samples were obtained 24 h after administration and the urine sample was collected between 12 and 24 h postdosing.

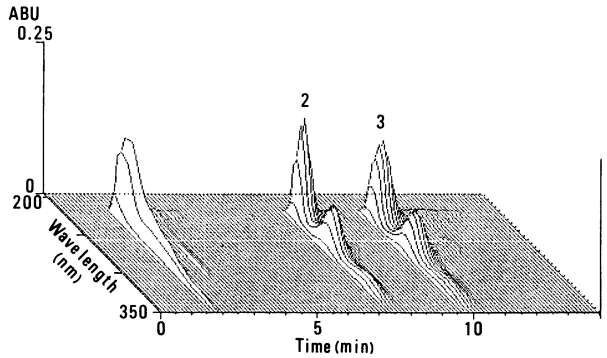


Fig. 2. Three-Dimensional Chromatogram Obtained from an Erythrocyte Sample of the Patient 24 h after Administration

Key: see Fig. 1.

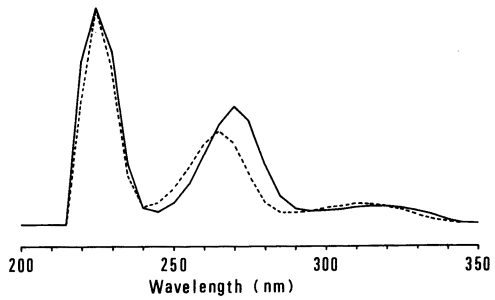


Fig. 3. UV Spectra of Hydrochlorothiazide Peak and Unknown Peak

——, hydrochlorothiazide; ------, unknown.

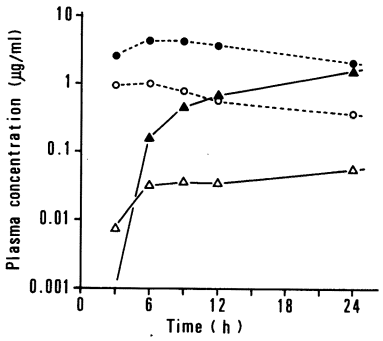


Fig. 4. Concentrations of Hydrochlorothiazide and Its Hydrolysate in Plasma and Erythrocyte from the Patient

---●---, HCT in erythrocyte; ---○---, HCT in plasma; —▲—, ACBS in erythrocyte; —△—, ACBS in plasma.

Fig. 2. The UV spectra of the HCT peak and the unknown peak are shown in Fig. 3. The unknown substance was identified by comparison of its HPLC elution profile and UV spectrum with those of authentic compounds; it was found to be a hydrolysis product of HCT, ACBS. It has been reported that commercial HCT tablets and bulk powder contain small amounts of ACBS as a degradation product of HCT.[6] Therefore, the amount of ACBS in tablets of the same lot as those administered to the patient was determined. The amount of ACBS was less than 0.4% of HCT. On the other hand, the amount of ACBS in the patient's urine collected up to 24 h was about 4.3% of HCT excreted in the urine.

As shown in Fig. 4, the HCT concentration in erythrocyte and plasma rose to a peak at 6 h postdosing and then declined slowly, although the ACBS concentrations in erythrocyte and plasma were still increasing at the end of the experimental period. The results indicate that ACBS is mainly formed *in vivo* by hydrolysis of HCT after administration and that the excretion rate of ACBS is slower than the rate of HCT hydrolysis to ACBS. The plasma concentration of HCT was about 10 times higher than that of ACBS. However, in erythrocyte, the concentration of ACBS was about equivalent to that of HCT. This fact shows that the affinity of ACBS to erythrocyte is much stronger than that of HCT.

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