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255. Akio Hoshi and Kazuo Kuretani*1 : Metabolism of Terephthalic Acid. III.*2 Absorption of Terephthalic Acid from Gastrointestinal Tract and Detection of Its Metabolites.

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The absorption of terephthalic acid (TPA) from gastrointestinal tract and its metabolites in urine were studied with radioactive TPA (carboxyl-^{14}C compound).

Terephthalic acid was absorbed mainly from stomach and small intestine and partly from caecum and large intestine.

The administered TPA was excreted almost quantitatively in urine within 24 hr. after the administration, and carbon dioxide as the cleavaged product was not found in the expired air.

Examinations by thin-layer chromatography and autoradiography technique failed to detect any metabolite in urine.

It was concluded that the administered TPA is not metabolized in body and almost all of it is excreted intact in urine.

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It was reported previously that the biological half-life of terephthalic acid (TPA) became longer in oral administration than in intraperitoneal injection.*2

In an effort to know the cause of this time prolongation, the TPA residue in gastrointestinal tract was examined by the chemical method,[1] but the amount of TPA which remained in the tract was too small to be detected owing to its coexistence with interfering materials which showed strong UV absorption at 240 mμ, the same as TPA.

In the present experiment, the radioactive TPA was used for ascertaining a small amount of the residue in the tract and the absorption sites of TPA.

In the previous report,[2] we noted that most of the administered TPA was excreted in urine, but it was not clear whether the TPA in urine was free or conjugated. Therefore, in the present study the form of excreted TPA was examined by the thin-layer chromatography.

Materials and Methods

Animals——Female rats of 200 g. (Wistar King-A) were used and the commercial diet (CA-1, Nihon CLEA Co., Tokyo) and water were offered *ad libitum*.

Materials——The radioactive TPA was the mixture of radioactive compound, a carboxyl-^{14}C compound (Daiichi Pure Chemicals Co.) and non-radioactive compound, 99.9% pure (Teijin Ltd.), and its specific activity was adjusted to 2 μc/mg. of TPA.

In the experiment for the detection of TPA metabolites in urine, non-radioactive componnd was also used.

Administraiton of TPA——Terephthalic acid was suspended at a ratio of 2% in 0.5% sodium carboxymethyl cellulose (CMC) solution using a glass homogenizer and the suspension, 85 mg. TPA per kg. of body weight, was administered orally through a stomach tube, while rats were fed 0.5% TPA supplemented diet for the detection of the metabolites in urine.

Treatment of Gastrointestinal Tract for the Absorption Experiment of TPA——Each group of five rats was killed respectively at 2, 4, 6, 8, 24 and 48 hr. after the radioactive TPA administration by bleeding from the carotid artery, and the gastrointestinal tract dissected into four parts : esophagus and stomach, small intestine, caecum and large intestine. Each of these was homogenized in 5 or 10% with 1*N* NH_4OH

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*2 Part II : Yakugaku Zasshi, **86**, 963 (1966).

1) A. Hoshi, K. Kuretani : *Ibid.*, **85**, 939 (1965).

2) *Idem* : *Ibid.*, **85**, 905 (1965).

and 0.1 ml. of the homogenate was used for radioactivity counting. Finally the radioactivity was converted into TPA mg.

Determination of Radioactivity in Gastrointestinal Tract——Radioactivity counting of the samples was performed originally by Herberg's method[3] and the counting efficiency was corrected by Baillie's method.[4] A "Packard Tri-carb 314 EX" liquid scintillation counter was used.

One-tenth ml. of homogenate and 3 ml. of hydroxide of Hyamine 10-X were taken into a vial, which was warmed in a water bath at 60° until the homogenate was dissolved. Fifteen ml. of scintillator solution, 0.05% dimethyl-POPOP and 0.5% PPO in toluene, was further added into the clear homogenate solution, the radioactivity of which was counted after neutralization of the solution. Benzoic ^{14}C-acid was used as the standard of radioactivity.

Collection of Carbon Dioxide in the Expired Air——Carbon dioxide in the expired air of an individual rat for 24 hr. was collected by Lifson's method.[5]

Detection of TPA and Its Metabolites in Urine by Thin-layer Chromatography——Thin-layer chromatography was used for checking the presence of TPA and its metabolites in urine.

An adsorbent, sillica gel GF_{254} (Merck) was mounted on a 50×200 mm. glass plate in 0.250 mm. thickness. The wet chromatoplate was left for 24 hr. at room temperature before use. The following three solvent systems (acidic, alkaline and neutral) were applied;

I; *n*-BuOH:AcOH:H_2O (60:20:20)[6]

II; 96% EtOH:H_2O:25% NH_4OH (100:12:16)[7]

III; *n*-BuOH:99.5% EtOH : H_2O (80:20:20)[8]

The sample of 50 to 100 μl. was spotted on the plate and developed ascendingly with a solvent.

A spot supposed as of TPA was identified by the following procedure; the sample and TPA or its sodium salt were placed each in a line, partly overlapping, on a plate, and were then developed with a solvent. When the both substances were identical, the two lines did not separate but overlapped each other.

The samples to be spotted were prepared and metabolites of TPA were detected on chromatograms according to the following procedures :

I) First fractionation of urine and detection of spots on chromatogram by color development and UV absorption.

1) Fractionation of urine : As the control (C), the urine of a basal diet fed rat was collected in a metabolism cage for 24 hr., then the diet was changed to the 0.5% TPA supplemented diet and the urine was recollected during the next 24 hr. for the treatment (T). These urines were fractionated a) for water soluble substance in neutral condition, b) for ethanol soluble substance in acidic condition and c) for ethanol soluble substance in acidic condition in the following ways :

a) Two ml. of urine was neutralized to pH 7.0 with 1*N* NaOH and concentrated into 0.5 ml. at room temperature under reduced pressure (fraction C-a and T-a).

b) Two ml. of urine was adjusted to pH 2.0 with 1*N* HCl and 8 ml. of EtOH was added. The mixture was centrifuged and the supernatant was evaporated to dryness at room temperature *in vacuo*. The dried sample was dissolved in 0.5 ml. of EtOH (C-b and T-b).

c) Two ml. of urine was adjusted to pH 2.0 with 1*N* HCl and 8 ml. of ether was added. The mixture was shaken well and ethereal layer was placed into another test tube and evaporated to dryness as described in b). The dried sample was dissolved in 0.5 ml. of ether (C-c and T-c).

2) Color development and UV-absorption of spots : Glucuronic acid and its relatives and amino acids and their relatives were detected by Partridge's method[9] with naphthoresorcinol (NR) and Patton's method[10] with ninhydrin (N), respectively. Intact TPA and other possible metabolites were indicated with UV-absorption at 254 mμ.

II) Second fractionation of urine and spot detection by autoradiography.

1) Fractionation of urine : Urine was collected for 2 hr. from 4th to 6th hour after the oral administration of radioactive TPA and fractionated stepwise as follows :

Two ml. of urine which contained about 2.8 mg. of TPA was adjusted to pH 2.0 with 1*N* HCl and extracted three times with 10 ml. portion of ether. The combined ethereal layer was evaporated to dryness at room temperature in vacuo. The dried extract was reextracted with 0.5 ml. of ether. The reextracted ethereal layer (fraction A) was transferred to another test tube and the residue was dissolved in 0.5 ml. EtOH (fraction B). The aqueous layer of the above extraction was adjusted to pH 6.0 with 15% NaOH and concentrated to 0.5 ml. at room temperature under reduced pressure (fraction W).

3) R. J. Herberg : Anal. Chem., **32**, 42 (1960).
4) L. A. Baillie : Int. J. appl. Rad. Isotopes, **8**, 1 (1960).
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6) K. Randerath : "Thin-layer Chromatography," 93 (1964), Academic Press, New York.
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8) L. Hough : Nature, **165**, 400 (1950).
9) S. M. Partridge : Biochem. J., **42**, 238 (1948).
10) A. R. Patton, P. Chism : Anal. Chem., **23**, 1683 (1951).

2) Autoradiography of spots : Thin-layer chromatogram was wrapped in a Saran film and placed on a X-ray film (Fuji 400 for X-ray). The film was developed and fixed by the ordinary method after 7 days exposure.

Results

Absorption of TPA from Gastrointestinal Tract——The absorption behavior of TPA in gastrointestinal tract, its absorption rate and the site of its absorption were investigated. Each of the residual amounts in four parts of the tract was measured and the results are shown in Table I.

Table I. Terephthalic Acid Contents in the Gastrointestinal Tract of Rat after a Single Oral Administration (85 mg./kg.)

Time after administration	TPA content (mg./rat)[a]				
	2	4	6	8	24
Stomach	6.76± 1.67	1.75± 0.62	0.43±0.39	0.08±0.01	0
Small intestine	2.59± 1.56	0.18± 0.15	0.05±0.04	0.04±0.04	0
Caecum	1.49± 1.06	3.12± 1.86	3.07±1.03	1.55±1.30	0
Large intestine	0.01± 0.01	1.11± 0.74	0.72±0.27	0.61±0.13	0
Total	10.85± 2.19	6.17± 2.03	4.28±1.31	2.28±1.39	0
Residue ratio (%)[b]	63.8 ±12.9	36.3 ±11.9	25.2 ±7.7	13.4 ±8.2	0

a) Mean value ± S.D. (5 rats)

b) Residue ratio (%) $= \frac{\text{Residual TPA in gastrointestinal tract (mg.)}}{\text{Administered TPA (mg.)}} \times 100$

The residual amount of TPA in the tract 2 hours after administration was 10.85 mg. per rat, which corresponded to approximately 64% of the administered TPA (17 mg./body). About 36% of the administered TPA gradually disappeared from the tract within 2 hours after administration. The TPA content of each part at this time was 6.76, 2.59, 1.49 and 0.01 mg. respectively, from upper to lower part. The TPA contents in the upper two parts abruptly decreased within the next 2 hours, that is, from 6.76 to 1.75 mg. in stomach and from 2.59 to 0.18 mg. in small intestine. On the other hand, the contents in the lower two parts increased from 1.49 to 3.12 mg. in caecum and 0.01 to 1.11 mg. in large intestine. The residue ratio of the tract at this time was about 36% and over half of the amount administered of TPA disappeared from the tract within 4 hours after administration.

After 6 and 8 hours TPA further decreased to 0.43 and 0.08 mg. in stomach and to 0.05 and 0.04 mg. in small intestine, respectively. In both caecum and large intestine,

Table II. Excretion of Terephthalic Acid after the Oral Administration of a Single Dose of 85 mg./kg.

Time (hr.)	Excreted TPA (%)[a]		
	Urine	Feces	Total
0～ 2	10.8±8.5	—[b]	10.8±8.5
0～ 4	33.6±7.1	—[b]	33.6±7.1
0～ 6	61.5±7.8	—[b]	61.5±7.8
0～ 8	82.1±7.5	0	82.1±7.5
0～24	93.5±7.6	3.3±2.1	96.8±6.4
0～48	93.8±7.6	3.3±2.1	97.1±6.4

a) Mean value ± S.D. (5 rats). b) No evacuation.

the contents gradually began to decrease after 4. The residue ratio of tract after 8 hours was only 13.4%. Twenty-four hours after administration, TPA was not found in any part.

Excretion in Urine and Feces——The TPA excretion in urine and feces was measured at the same time as described in the above, with results shown in Table II.

The integrated excretion ratios in urine 8, 24 and 48 hours after administration were 82.1, 93.5 and 93.8% and those in feces were 0, 3.3 and 3.3% of administered TPA, respectively. Almost all of the TPA was excreted within 24 hours in urine rather than in feces. These facts indicate that the administered TPA may be absorbed from the tract in a short period.

Radioactivity of Carbon Dioxide in Expired Air——To ascertain whether TPA was metabolized or not in the body, the carbon dioxide in expired air was collected for 24 hours after the TPA administration and production of radioactive carbon dioxide by the oxidative cleavage or decarboxylation was looked for. There was found no activity under this condition. The minimal detectable amount of TPA was 6 μg. per rat, which corresponded to 0.04% or less of the administered TPA. This result showed that TPA was not metabolized into carbon dioxide in the rat.

Metabolites in Urine——The presence of TPA metabolites in urine fractions was examined by thin-layer chromatography.

1) Examination with reagents: As shown in Fig. 1, there were two spots which were positive to naphthoresorcinol reagent and UV absorptive in the water soluble fractions, C-a, T-a, C-b and T-b. Rf values of the two spots were 0.00 and 0.50 respectively, which corresponded to glucuronic acid and glucuronolactone.

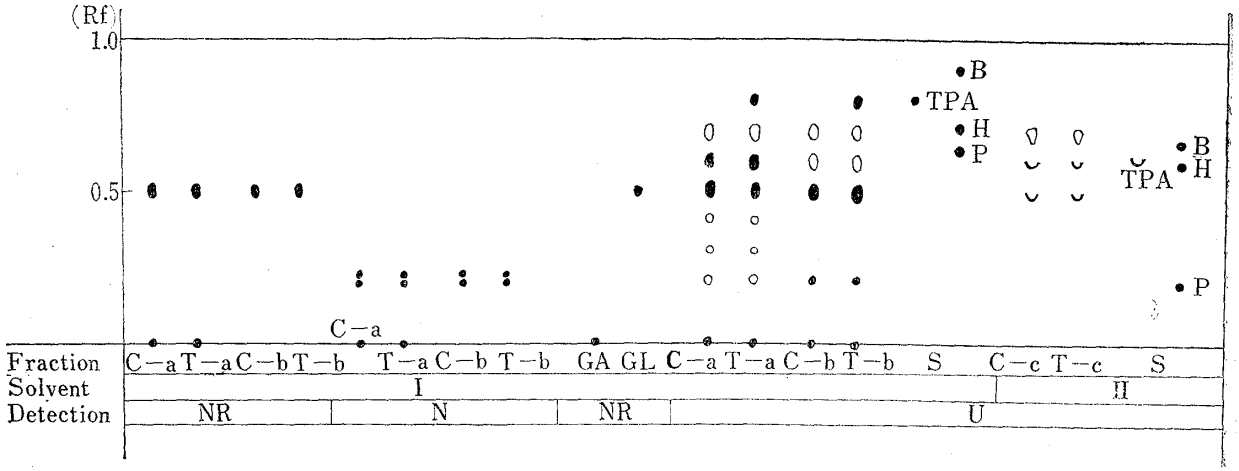

Fig. 1. Thin-layer Chromatogram colored with Specific Reagents in the First Examination

C-a: a-fraction of control
T-a: a-fraction of treatment
S: standard compounds
GA: glucuronic acid
GL: glucuronolactone
P: phthalic acid
TPA: terephthalic acid
H: *p*-hydroxy benzoic acid
B: benzoic acid
NR: naphthoresorcinol reagent
N: ninhydrin reagent
U: UV absorption

A ninhydrin positive and UV absorptive spot was also found at Rf 0.20 in the same fractions as described above. One spot was found only by UV absorption at Rf 0.80 in treatment (T) group but not in control (C) group, whose spot was identified as the intact TPA. These results showed that TPA was conjugated with neither glucuronic acid nor amino acids.

In ether soluble fraction, T-c and C-c, only three spots by UV absorption were found. However, there was no difference between two fractions. This result showed that TPA was not changed into phenolic compounds.

2) Examination by autoradiography : Because no metabolites were found in several fractions studied with chemicals as described above, radioactive compounds in the successive fractions were further examined.

Two ml. of urine containing 2.786 μg. of TPA was extracted three times in acidic-condition with each 10 ml. portion of ether. 2.777 μg., 99.7% of TPA, in urine was recovered in ethereal layer and only 0.9 μg., 0.3%, remained in aqueous layer. This ethereal layer corresponded to fractions A and B and water layer was fraction W mentioned in the item II-1. The results with these fractions by autoradiography and UV absorption are shown in Fig. 2.

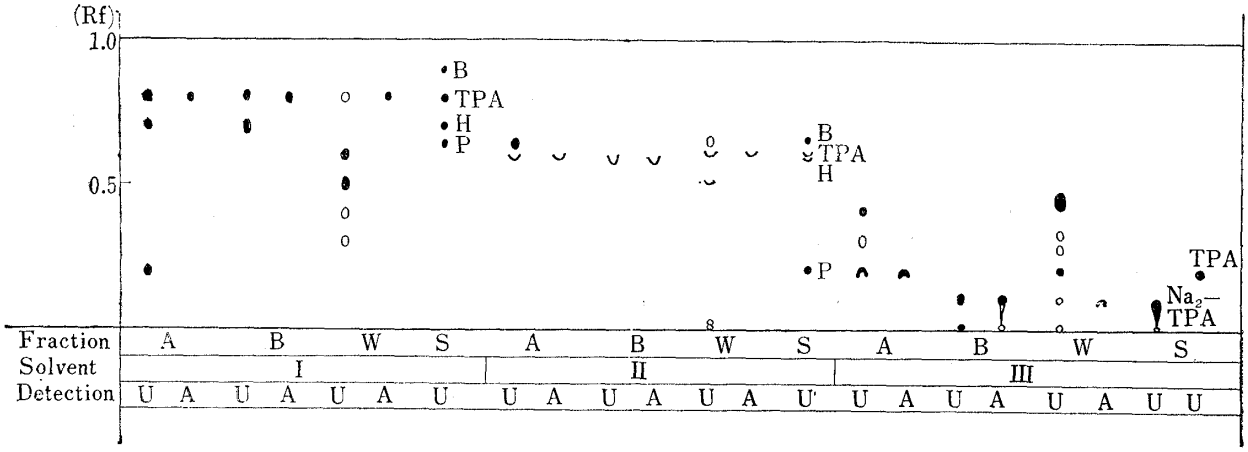

Fig. 2. Autoradiograph of Thin-layer Chromatogram in the Second Examination

P : phthalic acid
H : *p*-hydroxy benzoic acid
U : UV absorption
TPA : terephthalic acid
B : benzoic acid
A : radioactivity

Only one radioactive spot was found both in fractions A and W on chromatograms developed with three different solvent systems (I, II and III) and it was identified as the intact TPA.

Fraction B developed with either solvent I or II showed only one spot which was identified as TPA, while two spots were shown in the same fraction when developed with the neutral solvent (III). When TPA was added to fraction B and developed with the same solvent, TPA was overlapped on these unknown two spots and could not be differentiated from these spots. From this result, these unknown spots were concluded to be those of TPA itself, it being known that one compound is divided into several spots as artifact due to surrounding conditions, such as pH and salt concentration of the solution.[11]

Discussion

In the authors' previous works, it was shown that the excretion of TPA in urine was influenced by the administration route and it was suggested that TPA was absorbed slowly from the gastrointestinal tract.*[2]

In the present experiment, the TPA residue in the tract, the absorption rate and the absorption site were studied. TPA residue in the tract was rapidly lowered after administration; which suggested three possibilities, that is, decomposition, excretion in feces and absorption. However, the decomposition was out of question from the results of high recovery in urine and non detection of metabolites in urine and expired air. As the excretion in feces was only 3%, it could not be the main cause. It was therefore concluded that almost all of the administered TPA was absorbed from the tract in unchanged state.

11) K. Macek, I. M. Hais : "Paper Chromatography," 115 (1963), Academic Press, New York.

The maximum quantity in the latter two parts, caecum and large intestine, was 25.5% at 4 hours after administration and the TPA excretion in feces was only 3.3%. Consequently, it was calculated that over 22% of TPA in the latter two parts was absorbed from 4 to 24 hours after administration. Almost all of the rest, 70% or more, might be absorbed from the upper two parts, stomach and small intestine.

The metabolism of TPA in the dog was investigated by Porcher,[12] who reported that 75% of the administered TPA was excreted as free acid in urine and that no glycine conjugated TPA was found. His result shows that the TPA metabolite, if any, may be of a very small amount.

The metabolism of another benzenedicarboxylic acid was studied individually by Porcher[12] and Robinson.[13] Isophthalic acid (meta-dicarboxylic acid) and dogs were used in the former study and 4-hydroxyisophthalic acid and rats in the latter. The intact acids remained in the urines in both studies and none of the metabolites were found.

The present investigation carried out with the radioactive TPA must be capable of detecting very small quantities of the metabolites, and yet no metabolite was detected. From these data, it is possible to assume that the administered TPA cannot be metabolized in body but quantitatively excreted in urine.

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12) M. C. Porcher : Biochem. Z., **14**, 351 (1908).
13) F. A. Robinson, *et al.* : Biochem. J., **63**, 362 (1956).