Metabolic Fate of 1,2-Dibromo-3-Chloropropane (DBCP) in Rats

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Metabolic fate of 1,2-dibromo-3-chloropropane (DBCP) was investigated in male Wistar rats orally treated with 20 mg/kg of 3-¹⁴C-1,2-dibromo-3-chloropropane. Greater than 99% of the dose was absorbed, actively metabolized, and eliminated (84.9% of dose in 48 hr) in the urine, bile, and expired air. Radiocarbon expired was consisted mainly of radioactive carbon dioxide and also included a trace level of DBCP. Radiocarbon excreted in the urine was consisted of amphoteric and ninhydrinepositive conjugates (molecular weights, around 500) and 10 kinds of minor metabolites. Unchanged DBCP was not detected in the urine. Almost all of the tissues showed higher tissue radiocarbon levels than the plasma levels throughout the experimental term (28 days), and preferent distribution of radiocarbon was observed in the kidney and liver. Radiocarbon in tissues was classified into 3 groups as DBCP, organic-solvents extractable metabolites, and unextractable residue. DBCP consisted of only a part of tissue radiocarbon and was cumulative only in the adipose tissue. Organic-solvents extractable metabolites occupied the 2nd part and was considered cumulative. Predominant chemical form of tissue radiocarbon was organic-solvents unextractable substance typically observed in the kidney and liver.

INTRODUCTION

Haloalkanes are used for a variety of industrial, agricultural, and household uses. One member of the group, 1,2-dibromo-3-chloropropane (DBCP) have been used worldwide as a fumigant by virtue of its effective nematocidal activity. The chemical is reported having a potential to induce necrosis in the liver and kidney, atrophy of the testes, reduction of the sperm cell number, and development of abnormally formed sperm cells in rats.^{1–3)} In addition DBCP is listed up as a *in vivo* carcinogen in rats and mice.⁴⁾ Recently it was also documented that DBCP might sterilize human by reducing the number of the sperm cells.⁵⁾ In contrast to its active toxicological research, available data on its metabolic fate is only limited in soil⁶⁾ and no metabolic information is reported in animals. This paper presents the first information on metabolic fate of DBCP in animals.

MATERIALS AND METHODS

1. Chemicals

1,2-Dibromo-3-chloropropane-3-¹⁴C (¹⁴C-DBCP) prepared in New England Nuclear Co. was supplied from The Dow Chemical Company. Radiochemical purity was 94%, and specific radioactivity was 0.729 mCi/mmol. Pure nonlabeled DBCP was prepared in this laboratory from 3-chloro-1-propene and bromine. 1,2-Dibromoethane (EDB), 2,3-dibromopropionic acid, 3-bromopropionic acid, 3-chloropropionic acid, monobromoacetic acid, and glycolic acid were the products of Kanto Kagaku Co. Glutathion and glutathionedisulfide were obtained from Sigma Chemicals.

2. Animals

Male Wistar Imamichi rats of 9 weeks of age were used in this investigation (b.w., 270–290 g). They were treated with ¹⁴C-DBCP dissolved in olive oil (4.73 μ Ci/3.87 mg/ml) by

gastric intubation. The dose was 20 mg/24.5 μ Ci/kg, which is approximately 1/10 of rat oral LD₅₀¹⁾ and approximately 1/3 of the lowest single oral dose reported to induce an atrophy of the rat testes.²⁾ After certain periods 4 to 6 animals were sacrificed. The heparinized blood (3.9–4.1% of b.w.) was taken from the *vena cava candalis*, and representative 22 tissues were collected. Animal housing, collection of bile, urine, and feces from the rats with or without biliary cannulation and continuous blood collection from 6 animals to determine the time course of blood-¹⁴C levels were carried out in according to our routinely used method.⁷⁾ In this paper the blood volume was estimated as 6.0% of body weight in according to Cartland and Koch.⁸⁾

3. Analytical Procedure

3.1 Total radiocarbon analysis

Total radiocarbon in tissues, urine, feces, and bile was measured by liquid scintillation counting (Beckman LC-355) with (tissues and feces) or without (urine and bile) combustion (Packard Tri-Carb 306 Sample Oxidizer) as described in our paper cited above.

3.2 Extraction of tissue radiocarbon

The adipose tissue, blood cell, kidney, liver, plasma, and testes were homogenized with 3 volumes of methanol and centrifuged. The pellet was successively extracted 3 times by shaking with 5 volumes of 70% methanol, acetone, ethylacetate, and 80% methanol until any significant radioactivity was not found in the extracts. The extracts were combined and the residues were combusted prior to liquid scintillation counting.

3.3 Determination of DBCP in tissues

The above mentioned tissues of 12 animals orally treated with 20 mg/kg of non-labeled DBCP were collected 6 and 24 hr after dosing. They were individually homogenized in 200 ml of distilled water and steam distilled for 30 min with 5 ml of toluene. The toluene extract was dehydrated over anhydrous sodium sulfate and aliquotes of the extract were injected into ECD-gas chromatograph.

3.4 Radiocarbon analysis in expired air

Expired air was trapped through monoethanolamine in according to a routinely used method.⁹⁾ In addition, silica gel column (i.d.,

2.1 cm) packed with 20 g of silica gel G-60 (60–80 mesh, E. Merck)/cellite 545 (60–80 mesh, Wako) mixture (1:1, w/w) was also employed to trap radioactive volatile substances and exchanged every 3 hr. The column was eluted with 20 ml of acetone under a slight pressure by nitrogen gas. The eluant was radioassayed and injected into ECD-gas chromatograph to determine DBCP and some of its possible volatile metabolites. The mean recovery of ¹⁴C-DBCP through the silica gel column (fortification: 1 mg as vapor) was 96%. For the demonstration of radioactive carbon dioxide expiration, expired air was alternatively introduced into 100 ml of 4% barium hydroxide and 2 ml of monoethanolamine for 2 min in each hour. Radioactive precipitate of barium carbonate was collected by filtration and washed with 4% barium hydroxide and then with water. To determine the radioactivity in the precipitate, radioactive carbon dioxide was again generated by adding 6 N hydrochloric acid (100 ml) to the barium carbonate precipitate and introduced into gas-trapping tubes filled with 2 ml of monoethanolamine. The mean recovery of radioactive carbon dioxide by barium-hydroxide-trapping method was 48% of that by monoethanolamine trapping method.

3.5 Analysis of urinary metabolites

Buffered 0–48 hr urine (0.5 M potassium phosphate, pH 7.0, 20 ml) was steam distilled in a same manner as tissues described above and the toluene extract was injected into ECD-gas chromatograph. In another experiment partially deproteinized 0–48 hr urine (by 3 volumes of methanol) was analyzed by high performance liquid chromatograph. For further characterization of urinary metabolites, the urine (0–48 hr, 10 mls) was charged on Amberlite XAD-2 resin (Roam & Harce) columns (i.d., 5.4 cm $\times$ 20 cm) prepared by washing with 3 bed volumes of 0.05 N hydrochloric acid followed by successive and alternative 5-times washing with 3 bed volumes of water and methanol. The charged columns were eluted with 5 bed volumes of water until radioactive elution ceased and then with 3 bed volumes of methanol. Radioactive recovery was 90% (80% in aqueous eluant and 8.1% in methanol eluant). The aqueous eluant was evaporated and gel filtrated through Sephadex G-10 column. Radio-

active eluant was evaporated and processed to thin layer chromatography (*tlc*). The methanol eluant was also evaporated and spotted on thin layer films.

3.6 Chromatography

a) *gas chromatography (glc)*: Glc was conducted by using a Shimadzu GC-5AEFP gas chromatograph equipped with a ECD detector. DBCP and EDB was analyzed on a glass column (i.d., 3 mm × 2 m) packed with 5% OV-17 on Gas Chrome-Q (60-80 mesh, Nishio). The *glc* operating conditions for DBCP were: column temperature, 120°C; injection port and detector temperature, 200°C; flow rate of nitrogen gas, 60 ml/min. For the detection of EDB, column temperature was setted at 70°C, and flow rate of carrier gas was decreased to 40 ml/min. In these conditions retention times for DBCP and EDB were 1.4 and 1.1 min, respectively. The mean recovery of DBCP was 96% in all of the investigated tissues (fortification, 0.01 ppm) and no interference was noted in this analytical conditions. Detection limits of DBCP and EDB in tissues will be referred later in relative part of this paper.

b) *gel filtration and high performance liquid chromatography (hlc)*: Gel filtration was carried out by Sephadex G-10 column (i.d., 2.6 cm × 45 cm) swelled by distilled water. The column charged with 5 ml of sample was eluted with water at the flow rate of 0.5 ml/min and the eluant was collected by fraction collector (10 ml/fraction). Glutathione, glutathionedisulfide, and ¹⁴C-DBCP were eluted in the same column and assayed by the absorbance at 254 nm and liquid scintillation counting to estimate the molecular weight of DBCP metabolites. *Hlc* utilized Waters model 6000 A pump equipped with a μ Bondapack C18 column (i.d., 3 mm × 60 cm). Injection volume was 25 μ l and elution was carried out by methanol-water (3:1, v/v) at 1 ml/min of the flow rate. Radioactivity in the eluant was monitored by radioanalyzer equipped with a plastics scintillator (Aloka 601B and Fs-101). Operating conditions were: window setting, 20-55 division for ¹⁴C scanning; gain, ×1; time constant, 30 sec; range, 10 kcpm.

c) *tlc*: The methanol eluant (the 2nd bed volume) of urine through Amberlite XAD-2 resin column and radioactive eluant through

Sephadex G-10 were spotted on precoated silica gel GF-254 chromato films (0.25 mm gel thickness, E. Merck). Solvent systems were benzene-acetic acid (3:1, v/v) and butanol-acetic acid-water (6:3:1, v/v). Radioactive spots on *tlc* films were detected by autoradiography (non-screening X-ray film, Kodac AA54). For the detection of amino group and sulfur, ninhydrin reagent¹⁰⁾ was splayed and the radioactive spots scraped from the *tlc* films were submitted to sodium formate fusion test.¹¹⁾

RESULTS

1. Elimination of Radiocarbon

Radiocarbon in the gastrointestinal tract decreased to 1.65% of the initial dose in 24 hr with the biological half life ($T_{1/2}$) of 4 hr. Radioactive excretion was found in the urine, feces, bile, and expired air. Urinary excretion was 48% in 24 hr and cumulated to 51.4% in 14 days. Fecal elimination prolonged: it accounted for 14.4% in 24 hr and 22% in 14 days (Fig. 1). Expired air was trapped through silica gel column and monoethanolamine. Radioactivity trapped in silica gel column was 0.04% of dose in 48 hr, and in the same period 17.53%

Fig. 1 Radioactive excretion into urine and feces after a single oral dosage of ¹⁴C-DBCP (20 mg/kg).

●: urine, ○: feces, ○: urine + feces

Values are mean ± S.D. of more than 6 rats.

Table 1 Biliary excretion of radiocarbon after single oral dosage of ^{14}C -DBCP (20 mg/kg).

Duration	Cumulative radiocarbon excreted (% of dose)		
	Bile	Urine	Feces
3 hr	1.68 ± 0.14		
6	5.83 ± 1.45		
9	11.0 ± 1.85		
12	14.3 ± 2.93		
15	16.6 ± 2.33		
18	19.4 ± 2.62		
21	21.3 ± 3.35		
24	22.7 ± 3.80	19.2 ± 12.8	0.223 ± 0.052

Values are mean $\pm$ S.D. of 6 rats.

Fig. 2 Radioactive expiration after a single oral dosage of ^{14}C -DBCP (20 mg/kg).

○: cumulative value, ●: expiration rate
trapping agent: monoethanolamine
Values are mean $\pm$ S.D. of 4 rats.

of dose was trapped in monoethanolamine (Fig. 2). ECD-*glc* of silica gel column eluant showed that 0.03% of the dose was expired as DBCP in 48 hr. The rate of radioactive expiration (monoethanolamine trapping, % of dose/2 min) showed 2 peaks at around 3 and 9 hr after dosing. Demonstration of radioactive carbon dioxide expiration was carried out by introducing the expired air into barium hydroxide solution to yield radioactive barium carbonate. Approximately 83% of the radio-

carbon trapped in monoethanolamine was responsible for carbon dioxide. Biliary excretion was accounted for 22.7% of dose in 24 hr and appeared to prolong for more longer. In this experiment radioactive recovery in the feces of biliary cannulated rats was only 0.223% of oral dose indicating the active absorption of DBCP by rat gastrointestinal (Table 1).

2. Tissue Distribution of Radiocarbon

Radioactivity was detected in the blood soon after the dosage (15 min). The blood level increased gradually and reached plateau 3 to 12 hr after dosing. Residual radiocarbon in representative 22 tissues is shown in Table 2. The decrease of radiocarbon in all of the tissue examined exhibited biphasic pattern which could be divided into two phases at the 2nd day after dosage. The highest radiocarbon level was detected in the liver and the next in the kidney. The occupation of radiocarbon in these tissues was 5% in the liver and 1.3% of dose in the kidney at 6 hr after dosing. In the 1st phase (6 and 24 hr) the plasma level exceeded the blood cell level, and the liver, kidney, pancreas, thymus, and adrenal gland revealed higher radiocarbon residue levels than the plasma level. $T_{1/2}$ with the plasma in the 1st phase was 9.4 hr, and all of the tissues except the gastrointestinal tract showed longer $T_{1/2}$ s than that of the plasma. In the 2nd phase (later than the 2nd day after dosage) the blood cell level exceeded the plasma level, and similarly in the 1st phase almost all of the tissues examined showed significantly higher tissue radiocarbon levels and $T_{1/2}$ s than those

Table 2 Retention of radioactivity in male rats after single oral dosage of 3-¹⁴C-DBCP (20 mg/kg).

Tissue	ppm equivalents of 3- ¹⁴ C-DBCP							Biological half life	
	6 hr	1 day	2 day	4 day	7 day	14 day	28 day**	1st phase (hr)	2nd phase (day)
Blood	2.84±0.27	1.14±0.07	0.91±0.10	0.60±0.09	0.58±0.09	0.38±0.06	0.22	13.7	15.9
Plasma	3.67±0.55	0.98±0.12	0.62±0.10	0.24±0.03	0.13±0.03	0.05±0.01	<0.05	9.4	4.5
Blood cell	2.32±0.05	1.31±0.26	1.26±0.11	1.08±0.07	1.04±0.22	0.83±0.15	0.44	21.8	18.0
Liver	23.49±2.48 (5.05±0.33)*	13.5±2.43 (3.45±0.60)*	8.74±1.95 (1.81±0.25)*	5.31±0.48 (1.38±0.11)*	3.63±0.41 (0.78±0.12)*	1.46±0.17 (0.35±0.06)*	0.41	22.6	6.5
Kidney	28.2±6.08 (1.26±0.23)*	8.90±1.02 (0.42±0.03)*	5.79±0.87 (0.26±0.02)*	2.94±0.57 (0.14±0.01)*	1.77±0.12 (0.08±0.01)*	0.58±0.13	0.20	10.8	4.4
Lung	2.54±0.65	1.61±0.16	1.15±0.14	0.75±0.20	0.50±0.08	0.29±0.15		27.3	10.6
Brain	2.08±0.11	0.70±0.05	0.60±0.05	0.40±0.01	0.36±0.06	0.27±0.04	<0.11	11.4	12.6
Heart	2.27±0.28	0.98±0.14	0.75±0.03	0.64±0.23	0.56±0.07	0.30±0.05	<0.17	15.7	9.9
Spleen	3.07±0.90	1.74±0.25	1.13±0.12	0.79±0.18	0.71±0.08	0.43±0.06	0.34	22.0	19.2
Pancreas	6.97±0.94	1.98±0.37	1.18±0.15	—	0.62	0.30	—	9.9	6.5
Adipose tissue	4.52±1.41	1.46±0.58	1.00±0.32	1.16±0.63	0.57±0.04	0.55±0.34	0.43	10.9	22.0
Muscle	1.69±0.28	0.90±0.01	0.47±0.06	0.32±0.11	0.30±0.07	0.20±0.02	<0.16	19.6	13.7
Stomach	54.9±30.9	1.36±0.82	0.56±0.46	0.26±0.09	0.19±0.13	0.15±0.07	<0.15	4.1	10.3
Intestine	73.8±16.0	3.69±0.85	1.68±0.79	0.43±0.07	0.30±0.09	0.14±0.03	0.22		
Testes	2.25±0.31	0.81±0.10	0.65±0.08	0.34±0.05	0.41±0.03	0.20±0.05	<0.14	12.2	9.1
Seminal vesicle	2.35±0.76	1.27±0.26	0.96±0.16	0.83±0.20	0.55±0.05	0.21±0.01	<0.17	21.0	8.1
Epididymis	2.39±0.44	1.18±0.19	0.86±0.07	0.69±0.10	0.52±0.08	0.29±0.04	<0.22	17.6	8.2
Thymus gland	7.83±2.96	3.59±0.57	1.71±0.10	0.73±0.07	0.40±0.04	<0.21	<0.21	16.0	5.1
Adrenal gland	3.92±0.75	2.25±0.27	1.69±0.34	1.39±0.59	0.85±0.06	0.51±0.23	<0.36	22.5	6.9
Thyroid gland	2.56±0.52	1.32±0.22	1.17±0.40	1.10±0.23	<1.00	<1.00	<1.00	18.8	13.0
Pituitary gland	3.18±0.65	<2.80	<2.80	<2.80	<2.80	<2.80	<2.80	17.3	23.7
Remaining carcass	2.40±0.39 (8.43±1.43)	1.17±0.24 (3.93±0.73)	0.60±0.15 (2.14±0.48)	0.55±0.28 (2.15±1.11)	0.46±0.13 (1.76±0.56)	0.46±0.10 (2.08±0.40)	0.26 (1.29)	17.3	23.7

*: % of dose, 1st phase: within 2 days after dosing. Values are mean ± S.D. of 4 rats. **: N=2

of the plasma. The only exception was the kidney, which showed a similar $T_{1/2}$ as the plasma.

3. Characterization of Tissue Radiocarbon

Radiocarbon in selected tissues was repeatedly extracted with ethyl acetate, acetone, and methanol. The occupation of extractable radiocarbon ($ext-^{14}C$) in tissue radiocarbon was dependent on duration and varied amongst the tissues (Fig. 3A). The occupation of $ext-^{14}C$ reduced rapidly in the 1st phase and then gradually with the elapse of time in all the tissues examined. In the adipose tissue at least greater than 80% of the tissue radioactivity was extracted. In contrast, the occupation of $ext-^{14}C$ in the blood cell was the lowest amongst the investigated tissues (22% even 6 hr after dosing and less than 2.3% from the 2nd day on). The liver and plasma showed

Fig. 3 Extraction of tissue radiocarbon by organic solvents.

A: Extraction ratio, B: organic-solvents unextractable residue

—○—: adipose tissue, --○--: testes

—●—: kidney, --●--: liver

—●—: blood cell, --●--: plasma

Organic solvents: 70% methanol, acetone, ethylacetate, 80% methanol

Values are mean $\pm$ S.D. of 4 rats.

Table 3 Retention of extractable radiocarbon and DBCP in representative tissues of male rats after single oral dosage of 3- ^{14}C -DBCP or DBCP (20 mg/kg).

Duration	Plasma	Blood cell	Liver	Kidney	Testes	Adipose tissue
6 hr	1.07 $\pm$ 0.38 (1.0)	0.51 $\pm$ 0.13 (0.48)	7.70 $\pm$ 1.21 (7.2)	15.6 $\pm$ 4.2 (14.6)	1.56 $\pm$ 0.27 (1.40)	4.2 $\pm$ 1.3 (3.9)
1 day	[0.0027 $\pm$ 0.0121*] (2.48%)**	[0.0858 $\pm$ 0.0396*] (16.82%)**	[0.0335 $\pm$ 0.0118*] (0.435%)**	[0.0245 $\pm$ 0.0159*] (0.157%)**	[0.0090 $\pm$ 0.0043*] (0.58%)**	[2.136 $\pm$ 0.528*] (50.8%)**
	0.17 $\pm$ 0.01 (1.0)	0.088 $\pm$ 0.025 (0.53)	1.93 $\pm$ 0.42 (11.7)	1.81 $\pm$ 0.82 (10.9)	0.33 $\pm$ 0.05 (2.0)	1.26 $\pm$ 0.52 (6.1)
2 day	[0.0008 $\pm$ 0.0004*] (0.44%)**	[0.0018 $\pm$ 0.0008*] (2.07%)**	[0.0012 $\pm$ 0.0011*] (0.06%)**	[0.0012 $\pm$ 0.0004*] (0.07%)**	[0.0017 $\pm$ 0.0008*] (0.5%)**	[0.303 $\pm$ 0.388*] (24.0%)**
	0.052 $\pm$ 0.019 (1.0)	0.029 $\pm$ 0.003 (0.56)	0.86 $\pm$ 0.44 (16.5)	0.88 $\pm$ 0.14 (16.9)	0.21 $\pm$ 0.03 (4.0)	0.77 $\pm$ 0.26 (14.8)
4 day	0.019 $\pm$ 0.001 (1.0)	0.023 $\pm$ 0.010 (1.21)	0.24 $\pm$ 0.05 (12.6)	0.38 $\pm$ 0.16 (20)	0.11 $\pm$ 0.01 (5.8)	1.04 $\pm$ 0.58 (55)
7 day	0.009 $\pm$ 0.001 (1.0)	0.019 $\pm$ 0.007 (2.1)	0.18 $\pm$ 0.07 (20)	0.18 $\pm$ 0.01 (20)	—	0.42 $\pm$ 0.06 (47)

Values with no mark are extractable ^{14}C levels (ppm equivalents of 3- ^{14}C -DBCP) and means $\pm$ S.D. of 4 rats.

Values in parenthesis : tissue level/plasma level

[*]: DBCP level determined by ECD gas chromatography (means $\pm$ S.D. of 4 rats) [**]: % of extractable ^{14}C level. Radioactivity was extracted with 70% methanol, acetone, ethylacetate, and 80% methanol.

similar occupation which was in a middle range amongst the investigated tissues. From the testes greater than 35% of the tissue radiocarbon was extracted even on the 4th day, and the occupation of $ext-^{14}C$ was significantly ($p < 0.05$) higher than those of the kidney, liver, plasma, and blood cell. Table 3 shows the $ext-^{14}C$ levels expressed as ppm equivalent of ^{14}C -DBCP and also DBCP levels determined by ECD-*glc*. Relatively higher $ext-^{14}C$ levels were found in the adipose tissue, kidney, and liver as observed in total radiocarbon ($t-^{14}C$) analysis. In the 1st phase $ext-^{14}C$ level in the plasma exceeded the blood cell level as observed in $t-^{14}C$ analysis, however, in the 2nd phase the predominancy of the blood-cell $ext-^{14}C$ levels was not distinctive in contrast to that observed in $t-^{14}C$ analysis. All of the tissues revealed higher $ext-^{14}C$ levels than the plasma and also than the blood cell as typically observed in the kidney and liver. Furthermore the tissue/plasma level ratio increased with the elapse of time as critically observed in the adipose tissue. In regard to DBCP, it was present at trace levels and constituted only a part of tissue radiocarbon (less than 0.2%) except the adipose tissue. In the adipose tissue 50.8% of the $ext-^{14}C$ was responsible for DBCP, and the tissue showed significantly higher levels of DBCP ($p < 0.05$, in the 1st phase) than the plasma levels. Detection of EDB was tried in the adipose tissue and liver, however, the result was negative (< 0.005 ppm). On the other hand with respect to the unextractable radiocarbon ($unext-^{14}C$), its formation reached maximum as early as within 6 hr after dosage (Fig. 3B). At this point (6 hr) $unext-^{14}C$ occupied 3.35, 0.59, and approximately 0.54% of dose in the liver, kidney, and blood cell, respectively. The occupation of $unext-^{14}C$ in tissue radiocarbon reached maximum in 48 hr after dosage in all of the investigated tissues.

4. Metabolites in Urine

DBCP was not detected in the urine either by ECD-*glc* or *hlc*, and major metabolite was characterized as more polar than DBCP (elution volume: 8 ml for the major metabolite, 11 ml for DBCP) by *hlc*. When 0–48 hr urine was extracted with ether under basic (pH 14), acidic

Fig. 4 Sephadex G-10 elution pattern of urine sample.

A: blue dextran, B: glutathionedisulfide, C: glutathione, D: ^{14}C -DBCP

Mean of 3 experiments

Fig. 5 Tlc radioautograms of urine samples. A: Conjugate fraction through XAD-2 resin and Sephadex G-10 columns.

B-left: methanol eluant through XAD-2 resin column,

B-right: authentic chemicals

a: 3-bromopropionic acid, b: 3-chloropropionic acid, c: 2-bromopropionic acid, d: 2,3-dibromopropionic acid, e: monobromoacetic acid, f: glycolic acid

Solvent: buthanol-acetic acid-water (6 : 3 : 1, v/v) for A, benzene-acetic acid (3 : 1, v/v) for B

Tlc plate: silica gel GF254 (0.25 mm gel thickness)

Authentic chemicals were visualized by UV (254 nm), iodine vapor, and silver nitrate.

(pH 1), and neutral conditions, most of the radioactivity in the urine still remained in aqueous phase (91%). Gel filtration pattern of the aqueous eluant (80% recovery) through XAD-2 resin column showed 4 peaks, one of which was eluted in the high molecular weight (>600) fraction and occupied 18% of the charged radioactivity. The 2nd peak consisted of 56% of the charged activity or 43% of the radioactivity in 0-48 hr urine. The molecular weight of this main metabolite in the urine was larger than the parent chemical (MW , 236) and glutathion (MW , 306) and smaller than glutathionedisulfide (MW , 612) as shown in Fig. 4. *Tlc* of this conjugate fraction (fraction No., 12 and 13) showed that the conjugate was consisted of at least 4 radioactive and ninhydrin positive metabolites. On the other hand with respect to the methanol eluant through XAD-2 resin column (8.1% recovery), it showed 10 radioactive spots on *tlc*, 3 spots of which showed the same mobility as 2,3-dibromopropionic acid, 3-bromo- or 3-chloropropionic acid, and glycolic acid (Fig. 5). Sodium formate fusion test was tried to detect sulfur, however, the result was negative.

DISCUSSION

DBCP was readily absorbed from the rat gastrointestinal tract by the efficiency of greater than 99% as shown from the active biliary excretion over the fecal elimination and also from a trace level of fecal elimination by bilairy cannulated animals. Biotransformation of DBCP involved decarboxylation process of C-3 carbon and was active in rats as indicated by the active expiration of radiolabeled carbon dioxide soon after the dosing and by the radioactive composition in urinary excreta. However, the radioactive tissue retention was not so short as supposed but rather long. Radiocarbon absorbed did not homogenously distributed in the blood: in the 1st phase it distributed preferently in the plasma and in the 2nd phase in the blood cells. Although in this study the blood was not fully removed from the tissues and the tissue radiocarbon levels obtained might be slightly risen up from the net tissue levels by the effect of residual blood cell in tissues, radiocarbon due to ^{14}C -DBCP may be concluded to be cumulative in almost all of

the rat tissues. This cumulative property of radiocarbon was mainly a reflect of the peculiar metabolism of DBCP or of the formation of unusual substances unextractable by organic solvents which was typically found in the kidney and liver, toxicologically major target organs exhibit severe acute cellular necrosis.¹⁻³⁾ This substance is considered to be consisted mainly of proteins, because our further work indicated that it was trichloroacetic acid insoluble and macromolecular (MW , at least larger than 15,000 by gel filtration).¹²⁾ The 2nd chemical form was organic solvents-extractable metabolites which were also cumulative at least in the adipose tissue, blood cell, kidney, liver, and testes. DBCP itself consisted of only a part of tissue radiocarbon and was cumulative only in the adipose tissue. In this paper we found the major metabolite in the urine was a group of conjugates. They were characterized as follows: around 500 of the molecular weight, ninhydrin positive, eluted in aqueous phase through XAD-2 resin column, and unextractable by ether under any pH range. These properties suggest amphoteric nature of the conjugates and the occurrence of glutathione conjugates. Other minor metabolites were also found but were not well defined in this paper. Nevertheless, it is interesting that in this limited examination some radioactive spots showed similar mobilization to haloacids and glycolic acid on *tlc*. Because haloacids are known to have a potential to alkylate thiol, imidazol, and amino groups^{13,14)} which are universally found in proteins and nucleic acids.

The occurrence of organic-solvents unextractable residue and suggested formation of glutathione conjugates might be elucidated by the above mentioned interesting finding and further investigations are now proceeding.

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要 約

DBCP のラットにおける代謝

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1,2-dibromo-3-chloropropane-3- ^{14}C (^{14}C -DBCP) の雄ラットにおける動態を 20 mg/kg の 1 回経口投与系で調査した。吸収率は 99% 以上であり、活発に代謝されて 48 時間で投与量の 84.9% が尿, 胆汁, 呼気に排泄される。呼気中への主排泄形態は二酸化炭素であり, 微量成分として DBCP も検出された。尿中からは分子量約 500 と推定される両性的な, またニンヒドリン反応性を有する複数の抱合体が主代謝物として検出されたほか, 10 種の微量代謝物が検出された。一方 DBCP は尿からは検出されなかった。体内分布に関しては, 腎臓, 肝臓に偏在性が見られるが, その他の組織でも血漿レベルを上まわる ^{14}C 組織濃度が検出された。組織中 ^{14}C の存在様式を明らかにするため DBCP, 有機溶媒可溶性代謝物, 同不溶性残渣の三つに分けて主要組織中の ^{14}C を分析した。組織中 ^{14}C の主形態は有機溶媒不溶性の物質であり, 腎臓, 肝臓中でとくに顕著に認められる。第 2 の形態は有機溶媒可溶性代謝物であり蓄積的であった。DBCP は脂肪組織を除いて組織中 ^{14}C のごくわずかな部分を占めるにすぎず脂肪組織中でのみ蓄積的であった。