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Distribution of Terephthalic Acid in Tissues

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The terephthalic acid (TPA) in the tissues of rat was assayed with radioactive terephthalic carboxyl-^{14}C acid.

Two groups were fed the diet supplemented with 0.5% of TPA for one and three days respectively, and TPA content in each tissue was determined. No difference was found between the groups. TPA per unit weight or volume, in kidney and liver was higher than in plasma but in the rest of the tissues was lower (8—10 μg in plasma, 40—50 in kidney, 16—23 in liver, and 0.4—4.8 in the other tissues).

A single dose of TPA was distributed rapidly in the tissues within 2 hr, and the ratios of TPA contents in the tissues showed the same tendency as the above case. The maximum contents of TPA in the tissues were observed within 2 hr after administration, while in brain after 8 hr.

In the both cases, three-day feeding of the TPA diet and single oral administartion, a little amount of TPA was found after 24 hr only in several tissues. It already disappeared in more than half of the tissues.

The biological half-lives of TPAs in the tissues, 1.2—3.3 hr, were calculated from the result of single oral administration.

It is concluded that terephthalic acid is rapidly eliminated from the tissues and not accumulated in any of them.

The TPA content in plasma was assayed to be 5 μg/ml, while what was TPA in the tissues which may possibly contain it in a high concentration could not be assayed.[2,3] This fact is supposed to be due to the coexistent substances which may interfere with quantitative analysis of TPA.

Therefore, to overcome the above difficulty, the radioactive TPA was utilized for checking the microquantity of TPA in tissues and plasma. The counted radioactivity itself could represent the amount of contents in tissues and plasma, because TPA is not metabolized *in vivo* as reported previously.[4]

Materials and Methods

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

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

Administration of TPA——The experimental rats, fifteen in all, was divided into three groups, A, B and C. Group A was fed the mixture of 0.5% TPA and the basal diet for one day, group B was fed them for three days and group C was fed the basal diet for one day after the three-day feeding of the TPA diet was over. For single oral administration, animals were forcedly given TPA suspension, 85 mg. TPA per kg of body weight, by a stomach tube, which was the suspension of 2% TPA in 0.5% sodium carboxymethylcellulose solution prepared with a glass homogenizer.

Collection of Samples——Group A was killed just after one-day feeding, group B was killed after three-day feeding, and group C was killed one day after the three-day feeding of the TPA diet was over.

1) Location: *Tsukiji 5-Chome, Chuo-ku, Tokyo.*
2) A. Hoshi and K. Kuretani, *Yakugaku Zasshi*, **85**, 1007 (1965).
3) A. Hoshi, J. Takagi, and K. Kuretani, *Jap. J. Zootech. Sci.*, **37**, 115 (1966).
4) A. Hoshi and K. Kuretani, *Chem. Pharm. Bull.* (Tokyo), **15**, 1979 (1967).

Six groups each containing five animals were killed 2, 4, 6, 8, 24 and 48 hr, respectively, after single oral administration.

In both experiments, 4 ml blood was drawn from the vena cava with a heparinized syringe under light ether anesthesia and tissues were excised after the animals were killed by bleeding from the carotid artery. Blood cell and plasma were separated as described below. These samples were refrigerated and stored at −20°.

Treatment of Samples——As to blood cell fraction, 0.1 ml whole blood was taken in a screw cap vial, washed twice with 5 ml of 0.9% NaCl solution and centrifuged. Plasma was separated by centrifuging whole blood, 0.2 ml of which was used.

Tissues were cut finely with scissors, an aliquot of which was weighed as follows: 100 mg of liver, spleen, bone (femur) and salivary gland, and 200 mg of kidney, lung, heart, brain, muscle (thigh), pancreas, uterus, adipose (white) tissue and skin. For the rest of the tissues, whole organs were used because of their small quantities available, such as 30—50 mg of ovary, 9—13 mg of thyroid gland and 8—15 mg of pituitary gland.

Detection of Radioactivity——Radioactivity countings of the samples were performed originally by Herberg's method,[5] which was outlined in the previous report.[4]

Results

Terephthalic Acid Contents in Tissues under the TPA Diet Feeding

Terephthalic acid contents in the tissues were assayed in group A, B and C. The results are illustrated in Table I.

Table I. Distribution of Terephthalic Acid in Rat under TPA Diet Feeding

Group	TPA contents (μg/g or ml)[a]		
	A[c]	B[d]	C[e]
Plasma	9.77± 1.59	8.43± 5.22	0.04±0.02
Kidney	49.35±12.99	40.34±22.46	0.31±0.05
Liver	22.67± 8.78	15.77± 7.45	0.09±0.05
Brain	2.10± 0.31	1.87± 0.42	0.07±0.03
Skin	3.85± 1.45	4.75± 2.97	1.20±0.51
Lung	3.98± 1.95	3.88± 2.23	0
Pancreas	2.22± 0.85	4.64± 3.90	0.22±0.08
Spleen	1.36± 0.73	1.29± 0.69	0
Adipose tissue (white)	1.05± 0.68	2.28± 2.09	0
Heart	2.18± 1.07	1.77± 1.07	0
Muscle (thigh)	0.39± 0.12	0.84± 0.44	0
Bone (femur)	0.42± 0.27	0.51± 0.32	0
Blood cell[b]	0.66± 0.43	0.95± 0.84	0
Uterus	3.69± 1.45	3.66± 2.05	0.07±0.05
Ovary	2.7 ± 0.5	1.4 ± 1.0	0
Salivary gland	2.28± 0.74	1.36± 0.66	0.06±0.06
Thyroid gland	2.9 ± 0.8	2.8 ± 0.8	0
Pituitary gland	3.1 ± 1.5	3.0 ± 1.6	0
Adrenal gland	1.8 ± 0.6	1.3 ± 0.8	0

a) Mean value ± SE of each 5 rats
b) Corresponding to 1 ml of whole blood
c) They were fed the TPA diet for one day.
d) The were fed the TPA diet for three days.
e) They were fed basal diet for one day after three-day feeding of the TPA diet was over.

As compared with TPA content in plasma in group A, 9.77±1.59 μg/ml, that in liver and kideny was significantly high, 49.35±12.99 and 22.67±8.78 μg/g, and that in the rest of the tissues was low (3.98±1.95 μg/g in lung– 0.39±0.12 μg/g in muscle). Two days later, the content in plasma was 8.43±5.22 μg/ml and no difference was found between group A

5) R.J. Herberg, *Anal. Chem.*, **32**, 42 (1960).

TABLE II. Distribution of Terephthalic Acid after a Single Oral Dose of 85 mg/kg

Time (hr)	TPA contents (g/g or ml)[a]					
	2	4	6	8	24	48
Plasma	10.38± 1.74	6.75±2.05	2.96±0.32	2.38±0.37	0	0
Kidney	58.52±10.71	25.71±4.60	15.74±3.03	8.54±1.67	0.41±0.04	0
Liver	31.25± 2.88	12.96±2.18	8.14±1.40	5.13±0.56	0.13±0.04	0
Brain	0.98± 0.05	1.22±0.07	1.17±0.11	1.32±0.08	0.07±0.01	0
Skin	6.04± 1.33	2.91±0.45	2.14±0.42	1.90±0.29	0.06±0.04	0
Lung	4.19± 0.34	1.72±0.40	1.34±0.40	0.63±0.13	0	0
Pancreas	3.11± 0.37	1.06±0.09	0.63±0.16	0.38±0.05	0	0
Spleen	1.30± 0.16	0.47±0.09	0.34±0.11	0.22±0.04	0	0
Adipose tissue (white)	0.87± 0.22	0.45±0.05	0.36±0.09	0.16±0.02	0	0
Heart	2.53± 0.41	0.84±0.19	0.61±0.19	0.29±0.05	0	0
Muscle (thigh)	0.72± 0.11	0.31±0.05	0.24±0.10	0.09±0.01	0	0
Bone (femur)	0.41± 0.14	0.12±0.04	0.10±0.04	0	0	0
Blood cell[b]	0.43± 0.07	0.32±0.13	0.18±0.06	0	0	0
Uterus	5.67± 1.31	2.15±0.68	1.70±0.54	0.70±0.17	0	0
Ovary	4.4 ± 0.8	1.5 ±0.1	1.1 ±0.4	0.7 ±0.2	0	0
Salivary gland	3.16± 0.68	1.58±0.33	1.00±0.08	0.81±0.22	0	0
Thyroid gland	3.0 ± 0.3	2.0 ±0.3	1.4 ±0.3	1.0 ±0.4	0	0
Pituitary gland	3.1 ± 0.4	2.2 ±0.6	1.1 ±0.3	0.9 ±0.1	0	0
Adrenal gland	2.1 ± 0.2	0.9 ±0.2	0.5 ±0.1	0.2 ±0.1	0	0

a) Mean value ± SE of each 5 rats *b*) Corresponding to 1 ml of whole blood

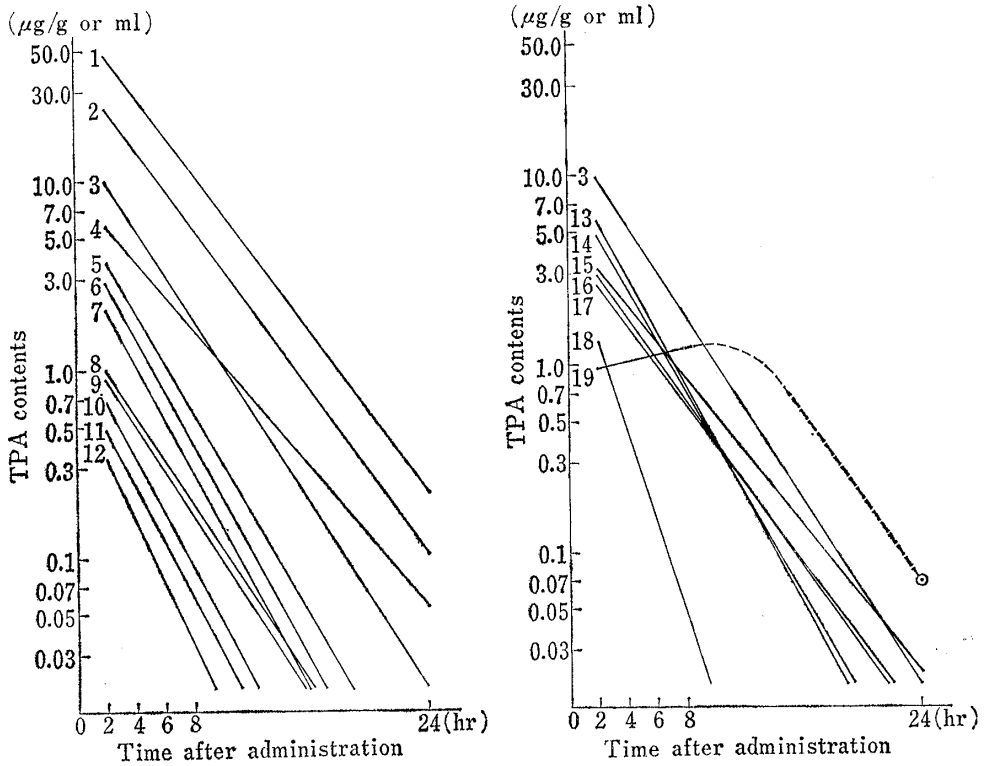

Fig. 1. Regression Lines of Tissue Telephthalic Acid after a Single Oral Dose of 85 mg/kg

1: Kidney 2: Liver 3: Plasma 4: Skin 5: Lung
6: Pancreas 7: Heart 8: Spleen 9: Adipose tissue 10: Muscle
11: Blood cell 12: Bone 13: Uterus 14: Ovary 15: Thyroid gland
16: Salivary gland 17: Pituitary gland 18: Adrenal gland 19: Brain

and B. Also the content in tissues did not change. These results show that the TPA fed with the diet is distributed widely in body, especially in liver and kidney, and is not accumulated at all in any of the tissues.

When animals were fed the basal diet after three day feeding of the TPA diet, TPA content in all their tissues decreased rapidly: 0.04 μg/ml in plasma, 1.20 μg/g in skin, and it had already disappeared from the other eleven tissues.

Terephthalic Acid Contents in Tissues after Single Oral Administration

The changes in the TPA content in body after single oral administration were traced from 2 to 48 hours after, because TPA in body was eliminated rapidly from the tissues. As shown in Table II, the highest content of TPA was found 2 hours after in plasma and the tissues except brain. The content in kidney 2 hours after was notably high, 58.52±10.71 μg/g, but in plasma it was about 10 μg/ml, both of which were almost the same as the respective values of the groups A and B above.

TPA content in the other tissues were also similar to that of the groups A and B.

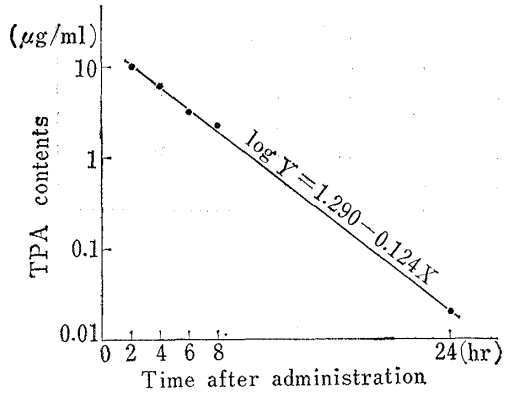

Fig. 2. Regression Line of Plasma Telephthalic Acid after a Single Oral Dose of 85 mg/kg

The regression lines for tissue TPA contents, log.–doses, by time after administration are shown in Fig. 1. These lines were linear and almost parallel mutually among plasma and the tissues except brain. The content in brain incerased slowly until 8 hours after administration, then decreased to zero by 48 hours.

The TPA regression line for plasma of Fig. 2 is expressed by the following equation;

$$Y=1.290-0.124X,$$

where Y is the log.–dose of plasma TPA (μg/ml) and X is the time after administration (hour).

The biological half–life of plasma TPA was 2.43 hours and its 95% confidence limits were 1.99 and 3.10 hours. The biological half–lives of tissue TPAs calculated from the regression lines in Fig. 1, were 1.2 to 3.3 hours. These facts mean that the TPAs in various tissues are rapidly eliminated from them and their regression rates are almost the same with each other.

Discussion

It has been previously ascertained by the chemical method that TPA in mammal's plasma was assayable to 5 μg/ml when one animal was used reciprocally for both the administration and its control.[2,6] On the other hand, TPA in tissues was not detected below 30 μg/g due to the coexistence of interfering substances.[3] In the present experiment, the radioactive compound was used for more accurate determination of TPA in body, whereby the TPA quantity can be calculated directly from the counted radioactivity itself, because TPA in body is excreted intact in urine and not metabolized *in vivo*.[4]

Finally, the detection limit could be lowered to 0.03 μg/g or ml.

The TPA content in an individual tissue of group B was almost the same as that of group A and the daily accumulation was not recognized in any tissue. There was about 10 μg/ml. in plasma, equal to the content suggested from the experiment of fowl fed TPA diet.[3]

This low level in plasma might be caused either by the rapid transference into tissues and the excretion in urine. The changes in TPA content in brain were characterized by slow increase until 8 hours after administraion, different from the other tissues in which it reached the maximum within 2 hours. TPA may be passed slowly through the blood–brain barrier.

6) A. Hoshi, J. Takagi, R. Yanai, and K. Kuretani, *Yakugaku Zasshi*, **86**, 963 (1966).

The disappearance of TPA from tissues was rapid. Even in kidney which contained the highest quantity of TPA, its content 24 hours after three-day feeding of the experimental diet and single oral adminisatration of TPA was only 0.31 μg/g and 0.41 μg/g each.

Biological half-life of TPA was from 1.2 to 3.3 hours in several tissues and 2.43 hours in plasma. These values were almost the same as what was obtained in the previous work about the excretion rate in ruine.[6)]

It was concluded that terephthalic acid was eliminated rapidly from tissues and plasma and not accumulated in any tissue.

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