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Distribution, Percutaneous Absorption and Excretion of Isocyanuric Acid

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¹⁴C-Isocyanuric acid given orally to rat distributed rapidly, showing higher radioactive concentration in blood, liver and kidney, and the peaks of concentration in all of organs and tissues were observed at 30 min. after dosing. Thereafter, the radioactivities decreased rapidly. After repeated oral administration, a similar result was obtained, so any accumulating effect was not noted.

The percutaneous absorption of the labeled compound was so scarce that no radioactivity was found in many blood samples taken intermittently after the application on rat skin.

The clearance of the compound after oral administration was also very fast and the main excretion route was urine. The excretion to expiration, bile and feces was very little. More than 99 % of the urinary radioactive substance was identified to be intact compound.

Keywords—isocyanuric acid ; oral administration ; distribution in rat ; absorption to rat ; percutaneous absorption ; excretion to expiration

Introduction

Isocyanuric acid (2,4,6-triazinetriene, ICA) and isocyanurate have been used in swimming pool as stabilizing agent for chlorine, hypochlorous acid and hypochlorite ion. Its chlorinated product, chlorinated isocyanurate, is known as one of the chlorine disinfectants.

According to the literatures on toxicity of cyanurate,^{2,3)} this compound has rather low acute and chronic toxicity. However, owing to the fact that this compound is used in the water of swimming pool, oral and percutaneous introduction to body should be considered.

In this paper, the distribution in rat after single and repeated oral administration, percutaneous absorption, excretion to expiration, bile, urine and feces, and determination of urinary metabolite were presented. For the radioactive tracer, an aqueous solution of ¹⁴C-ICA was prepared by dissolving ¹⁴C-cyanuric chloride (2,4,6-trichloro-s-triazine) in water, because it is acknowledged that cyanuric chloride is readily decomposed to cyanuric acid (2,4,6-s-triazinetriol) and hydrochloric acid⁴⁾ and cyanuric acid exists in carbonyl form^{5,6)} in neutral aqueous solution.

Furthermore, a result of experiment on

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the biological decomposition of the urinary excreted compound was also described together with some discussion.

Material and Method

1. **Animal**—Wistar strain male rats, 12 weeks old weighing 207 ± 13 g and 5 weeks old weighing 144 ± 11 g were used for single and repeated oral dosing respectively. For the experiments on percutaneous absorption, excretion to expiration and bile, 15 weeks old rats of the same strain weighing 240 ± 8 g were used.

2. **Material for Dosing**— ^{14}C -2,4,6-cyanuric chloride (New England Nuclear Corp.) was dissolved in water by heating at 90° for 4 hours to convert to ^{14}C -2,4,6-ICA; and the solutions of required concentration were prepared by an addition of carrier solution. The radiochemical purity of the heated solution was verified to be pure quality by thin layer chromatography and isotope dilution method using the reference standard of ICA and cyanuric chloride.

3. **Distribution and Excretion to Urine and Feces after Single Oral Administration**—To 6 groups of 3 rats, $50 \mu\text{Ci}/0.410 \text{ mg/ml/kg}$ of ^{14}C -ICA solution was given orally with stomach catheter, and at appropriate intervals after the dosing each group was dissected to take organs and tissues.

At the dissection, blood was sampled at first with heparinated syringe from vein (vena cava cranialis) prior to taking out the organs and tissues. The gastrointestinal tract was divided to stomach, upper part of small intestine, lower part of small intestine, large intestine and their contents.

The preparation of samples for measuring radioactivity was carried out as follows; The whole organ or tissue, or a part (about 40 mg or less) of blood and large organs such as brain, liver was weighed in vial, 1.0 ml of Soluene-350 (Packard Instrument Company, INC) was added, allowed to stand at 40 – 50° in a thermostat for 1–2 days to dissolve the material, and 15 ml of toluene base scintillator (DPO 5 g, POPOP 0.3 g, toluene 1 l, acetic acid 2 ml) was added.

The feces collected by the cage fitted with

urine-feces separator was dried in refrigerator, and after crushing, a part of it (about 40 mg) was incinerated in an automatic sample combustion system (Aloka, ASC-111). The generated $^{14}\text{CO}_2$ was absorbed to toluene base scintillator mixed with ethanolamine.

The urine collected was diluted in a volumetric flask, 1.0 ml was placed in vial, and 15 ml of toluene base scintillator mixed with a solution of nonionic surface active reagent (Nissan Nonion NS-210) (6:4) was added.

To observe the distribution in blood, a part of the sampled blood (about 20 mg) was taken in vial, and the remainder was separated to serum and red cells by ordinary method. A part of serum (about 40 mg) and red cells (about 20 mg) were placed in vials and dissolved in toluene base scintillator after the Soluene-350 treatment.

4. **Distribution after Repeated Oral Administration**—Four groups of 3 rats were given orally $12 \mu\text{Ci}/0.410 \text{ mg/ml/kg}$ of solution twice a day—at 9.00 am and 6.00 pm—repeatedly for 20 days. The urine and feces were collected every day and at 30 min, 1, 3, 12 hours after last dosing each group was dissected. The organs, tissues and excreta were treated under the same procedure as section 3.

5. **Percutaneous Absorption**—A piece of duplicated gauze about 5×9 cm wide adsorbed $56 \mu\text{Ci}/0.497 \text{ mg}/0.8 \text{ ml}$ of the solution was attached closely to the back skin of 3 groups of 3 rats whose back hairs were removed beforehand by hair clippers for animal use. The gauze piece was covered with a polyvinyl chloride sheet and fixed with a gummed tape. At 6, 9 and 12 hours after application, the gauze piece of each group was removed, and the applied area of skin was wiped thoroughly with a piece of gauze containing water, then at 21 hours after the application all groups of rats were sacrificed. Meanwhile, blood and urine were sampled at every 3 hours. After sacrifice, the applied area of skin was picked out, and the surface was rinsed repeatedly with about 100 ml in total of water.

About 20 mg of blood and about 40 mg of cut skin were weighed in vials, treated with Soluene-350

and mixed with 15 ml of toluene base scintillator. The urine and washings were treated under the same procedure as described in section 3. The gauze pieces used for application and wiping the area of skin were separately cut to very small pieces and a part of them was placed in vial containing 20 ml of dioxane base scintillator (DPO 5 g, POPOP 0.4 g, naphthalene 50 g, dioxane 1 l), and the radioactivity was measured after standing for several hours to dissolve thoroughly the substance adhered to gauze.

6. Excretion to Expiration—0.25 and 0.51 ml of the solution of 50 $\mu\text{Ci}/0.410\text{ mg/ml}$ were given to 2 rats orally, and 0.3, 0.4 and 0.6 ml of the solution of 12 $\mu\text{Ci}/0.410\text{ mg/ml}$ were injected intravenously to 3 rats.

Just after dosing, the animal was placed in a radiorespirometry sampling system (Natsume, KN-0070) for measuring $^{14}\text{CO}_2$ in expiration. The air was introduced to the apparatus at the flow rate of 200 ml/min, and CO_2 in expiration was absorbed in 100 ml of a mixture of ethanolamine and methylcellosolve (2:1), changing the bottle of absorbing mixture to fresh one at every 2 hours. The surface of urine in reservoir in the sampling system was covered with toluene layer in order to prevent the contamination of expiration by urine. Then, 2 ml of the absorbed mixture was placed in vial, and the sample solution for measuring radioactivity was prepared by addition of 15 ml of toluene base scintillator.

7. Excretion to Bile—Three rats were anesthetized with ether. The bile duct was surgically exposed by a mid-line incision and cannulated with a polyethylene tubing ($\phi=1\text{ mm}$). The tube was then tied up and the abdomen was closed. After recovery from anesthesia, each rat received 56 $\mu\text{Ci}/0.96\text{ mg}/2\text{ ml/kg}$ of ^{14}C -ICA solution by a single oral administration. After that, bile was collected directly from the tube to vial. The vial was replaced with new one at every 30 min. until 12 hours after dosing, thereafter one more vial was enough to collect the bile until 24 hours. The measurement of radioactivity was carried out in the same manner as the case of urine.

8. Instrument—A liquid scintillation counter

(Aloka, LSC-651) and a thin layer chromatoscanner (Aloka, JTC-203) were employed for measurement of radioactivity.

Result

1. Distribution

(1) Distribution after Single Oral Administration—The radioactive concentration in major organs and tissues at 15, 30 min, 1, 3, 6 and 12 hours after the oral administration of ^{14}C -ICA was shown in Table I, and in Table II was shown the distribution of radioactivities mainly in gastrointestinal tract, expressed in % of dose.

The radioactivity was distributed very rapidly and rather evenly throughout the whole body, and higher radioactive concentration was found in blood, liver and kidney, showing their peaks at 30 min. after dosing as well as in other organs and tissues, and thereafter decreased fast (Table I). As shown in Table II, a rapid decrease of radioactivity] was also observed in the gastrointestinal tracts and their contents. At 6 hours after dosing, the radioactivity was scarcely noted except the content of stomach, in which radioactivity was also almost disappeared at 12 hours after dosing, and these findings were in good agreement with the result shown in Table I.

(2) Turn Over of Radioactivity in Blood, Liver and Kidney after Single Oral Administration—The radioactive concentration in blood, liver and kidney was particularly measured in order to know the rapidness of clearance and the result obtained at several intervals after single oral administration of 25 $\mu\text{Ci}/0.205\text{ mg/ml/kg}$ of ^{14}C -ICA was shown in Table III and Fig. 1. In the Table, the relative concentration to blood was presented in parenthesis for comparison. The radioactive concentration in kidney was always over 3 times higher than that in blood at any

TABLE I. Radioactive Concentration in Organs and Tissues after Single Oral Administration (dpm/g)^{a)}

Organ and tissue	Time after administration					
	15 min	30 min	1 hr	3 hr	6 hr	12 hr
Blood	81410	99080	60380	13320	1050	370
Brain	2750	6550	3870	2250	1010	180
Spinal cord	3110	6650	5150	2380	860	70
Sciatic nerve	8650	32840	13720	9250	1680	210
Pituitary	30940	82350	35230	6430	4500	0
Thyroid	25070	42750	17300	4710	1680	450
Salivary gland	38870	68460	32690	8390	1050	310
Thymus	33360	62900	33400	7200	1070	220
Heart	38800	64110	34250	8670	950	60
Lung	55310	79170	40870	11850	1110	310
Liver	78660	97390	48820	11330	2330	490
Spleen	39610	71830	35040	13850	1290	560
Pancreas	30310	86120	46870	11010	1920	640
Adrenal	40040	75130	35580	9870	2020	480
Kidney	440430	444200	300340	123620	8940	330
Testis	11580	17080	15150	8490	2300	510
Epididymis	15970	42320	32310	12510	2960	650
Seminal vesicle	26500	61520	26160	12470	1890	320
Bone marrow	33080	57130	36340	9110	2090	560
Muscle	19670	42300	27680	7770	620	70

^{a)} Each value represents the mean of 3 rats.

TABLE II. Distribution of Radioactivities in Gastrointestinal Tract and Its Content after Single Oral Administration (% of dose)^{a)}

Tissue	Time after administration					
	15 min	30 min	1 hr	3 hr	6 hr	12 hr
Stomach						
wall	2.72	1.45	2.07	0.27	0.06	0.02
content	42.68	10.19	12.21	5.65	0.42	0.01
Upper small intestine						
wall	2.61	1.13	0.74	0.15	0.04	0.01
content	8.87	3.68	1.36	0.22	0.04	0.00
Lower small intestine						
wall	0.85	0.81	0.99	0.22	0.02	0.01
content	1.46	1.82	1.86	0.09	0.18	0.04
Large intestine						
wall	0.47	0.69	0.23	0.09	0.02	0.00
content	0.21	0.37	0.19	0.09	0.18	0.03
Other parts of body ^{b)}	40.13	70.38	61.26	29.96	12.26	9.63
Excreted	—	9.43	19.09	63.26	86.78	90.25

^{a)} Each value represents the mean of 3 rats.

^{b)} Estimated from relating data.

TABLE III. Radioactive Concentration in Blood, Liver and Kidney after Single Oral Administration (dpm/g)^{a)}

Organ and blood	Time after administration						
	10 min	20 min	30 min	45 min	1 hr	3 hr	6 hr
Blood	19320 (1.00)	39740 (1.00)	38910 (1.00)	33770 (1.00)	22840 (1.00)	4340 (1.00)	1470 (1.00)
Serum	26250 (1.37)	51200 (1.30)	43000 (1.10)	37070 (1.11)	25620 (1.13)	5550 (1.32)	1620 (1.03)
Red cell	2330 (0.12)	5730 (0.14)	5400 (0.14)	5650 (0.17)	1180 (0.05)	160 (0.03)	170 (0.11)
Liver	22780 (1.18)	36760 (0.93)	32010 (0.82)	28950 (0.85)	18090 (0.78)	4840 (1.11)	1470 (1.15)
Kidney	72140 (3.75)	143200 (3.59)	136580 (3.53)	105520 (3.18)	68960 (3.14)	22790 (4.91)	5640 (3.47)

a) Each value represents the mean of 3 rats. Value in parenthesis is relative concentration calculated by the equation.

$$\text{Relative concentration} = \frac{\text{dpm/g of blood component or organ}}{\text{dpm/g of blood sampled at each same time as above}}$$

Fig. 1. Turn Over of Radioactivities in Blood, Liver and Kidney after Single Oral Administration

—○— blood, —△— liver, —●— kidney.
Biological half time: blood 65 min, liver 70 min, kidney 65 min.

time investigated, while liver showed almost the same level as blood. The major part of radioactivity in blood was distributed to serum, showing about 10 times higher value than red cells throughout the experimental time.

In Fig. 1, the radioactivities in whole blood, liver and kidney were expressed in % of dose. The values showed their peaks at 20 min after dosing as well as the concentrations, and thereafter decreased remarkably. The biological half time estimated from the

curves in Fig. 1 was 65 min for blood and kidney and 70 min for liver.

(3) Distribution after repeated Oral Administration—The radioactive concentration in organs and tissues at several time intervals after the last dosing of repeated, twice a day for 20 days, oral administration of ^{14}C -ICA was shown in Table IV. and the distribution of radioactivities in gastrointestinal tract expressed likewise to the case of single dosing was presented in Table V.

The radioactive distribution in the animals of repeated administration showed similar pattern to the single dose group, namely, excepting the gastrointestinal tract and its content, higher distribution was seen in blood, liver and kidney. The radioactivity in all organs and tissues showed their peaks at 30 min after dosing, decreased thereafter and was not detected practically at 12 hours after dosing. There was noted no difference between the results of distribution after single and repeated dosing, therefore, any accumulating effect of repeated administration was not observed.

TABLE IV. Radioactive Concentration in Organs and Tissues after Repeated Oral Administration (dpm/g)^{a)}

Organ and tissue	Time after last administration			
	30 min	1 hr	3 hr	12 hr
Blood	15840	13260	2980	360
Brain	1320	1020	490	210
Spinal cord	1320	1190	430	200
Sciatic nerve	1490	2340	1420	410
Pituitary	12170	4500	1040	840
Thyroid	5610	2360	270	450
Salivary gland	9250	7210	10590	320
Thymus	9680	7390	1280	340
Heart	10470	8380	1470	270
Lung	13930	9780	1570	320
Liver	15940	11550	2290	520
Spleen	9630	7770	1120	350
Pancreas	9380	7390	1360	390
Adrenal	11280	8720	1410	610
Kidney	76970	58440	9310	780
Testis	3600	2890	1280	350
Epididymis	7720	6130	1640	330
Seminal vesicle	8430	5100	2470	460
Bone marrow	11600	7730	1420	440
Muscle	5950	5740	1130	260
Adipose tissue	3110	2630	510	660

a) Each value represents the mean of 3 rats.

TABLE V. Distribution of Radioactivities in Gastrointestinal Tract and Its Content after Repeated Oral Administration (% of dose)^{a)}

Tissue	Time after last administration			
	0.5 hr	1 hr	3 hr	12 hr
Stomach				
wall	2.55	1.94	0.93	0.02
content	39.93	23.13	5.97	0.01
Upper small intestine				
wall	1.82	0.91	0.30	0.02
content	4.87	1.60	0.32	0.01
Lower small intestine				
wall	1.13	0.66	0.14	0.02
content	5.02	2.49	1.44	0.06
Large intestine				
wall	0.22	0.17	0.05	0.01
content	0.15	0.19	0.09	0.11
Other part of body ^{b)}	25.08	43.17	47.74	7.61
Excreted	19.27	25.74	43.02	92.13

a) Each value represents the mean of 3 rats.

b) Estimated from relating data.

2. Percutaneous Absorption

To investigate the percutaneous absorption, only the rinsed skin of applied area, and blood and urine sampled repeatedly were measured, because blood and urine were regarded to be able to indicate the absorption, considering the thicker distribution to blood and prominent excretion to urine after oral administration. The radioactivity in the pieces of gauze used for application and wiping together with the rinsing water was also measured.

The result obtained after the application of 56 $\mu\text{Ci}/0.497 \text{ mg}/0.8 \text{ ml}$ of test solution to the back of rat for 6, 9 and 12 hours was presented in Table VI. Although the radioactivities remained in the rinsed skin were in 1–3 % of applied dose, no significant radioactivity was detected in any of many blood samples taken at every 3 hours from the beginning of application to 21 hours later. Moreover, the total radioactivities in urine samples taken at the same time as blood were less than 0.01 % of dose. Therefore, it was concluded that the percutaneous absorption, namely the transference through skin, of ^{14}C -ICA might be very scarce.

TABLE VI. Percutaneous Absorption of Radioactivity at 21 Hours after Application to Rat Skin (% of dose)

Length of applicat. time (hours)	Rinsed skin	Blood ^{a)} samples	Total ^{b)} urine	Pieces of gauze and rinsing water
6	2.24 $\pm$ 0.60	Nil ^{c)}	0.008 $\pm$ 0.006	85.29 $\pm$ 0.60
9	2.71 $\pm$ 1.14	Nil	0.007 $\pm$ 0.001	82.02 $\pm$ 1.14
12	1.24 $\pm$ 0.23	Nil	0.009 $\pm$ 0.006	85.48 $\pm$ 0.23

a) Many samples taken at every 3 hours during the experimental time.

b) Total of urine samples taken at the same times as blood.

c) Not detected.

3. Excretion

(1) **Excretion to Expiration**—The radioactivity in expiration collected in every 2 hours after the administration was shown in Fig. 2, being expressed in % of dose. After oral administration, a very few amount of radioactivity was expired continuously up to 10 hours after dosing, showing a gently-sloping peak at 4–6 hours after dosing, and only about 0.1 % of dose in total was excreted in 10 hours. In the case of intravenous injection, the expired radioactivity increased rapidly up to 6 hours after injection and thereafter decreased also rapidly. The total excretion in 12 hours was accounted to be about 1.2 % of dose, and there was no difference relating to dose as well as the case of oral dosing.

(2) **Excretion to Bile**—For the experiment on excretion to bile 3 rats were used, but the results were almost identical to each other. So the representative result of the excreting pattern of one rat was shown in Fig. 3.

The relatively much excretion to bile was observed mainly in the first hour after oral

Fig. 2. Excretion of Radioactivities to Expiration after Oral and Intravenous Administration

Oral administ. : —▲— 0.25 ml/rat.
 —△— 0.51 ml/rat.
 Intravenous inject. : —●— 0.3 ml/rat.
 —○— 0.4 ml/rat.
 —×— 0.6 ml/rat.

Fig. 3. Excretion of Radioactivity to Bile after Oral Administration

dosing, and decreased with time lapse. After 12 hours, the radioactivity in bile became inconsiderable, and the total excretion rate to bile in 24 hours after dosing was only 0.50–0.10 % of dose.

(3) **Excretion to Urine and Feces**—The cumulative excretion rates at several intervals after single oral administration were presented in Table VII. The major part of given radioactivity was excreted very fast to urine, namely, a remarkable excretion was found as early as 30 min. and about 90 % of dose was excreted in 12 hours after dosing. While only about 1.5 % of dose was found in feces in the same period.

On the other hand, every day excretion to urine and feces, expressed in % of a dose on each foregoing day, was measured during the repeated oral administration. The radioactivities were excreted to urine and feces by almost constant rate every day, and the mean values of excretion rate during 20 days were $94.5 \pm 3.1\%$ and $3.28 \pm 1.03\%$, and the

TABLE VII. Cumulative Excretion Rate after Single Oral Administration (% of dose)^{a)}

Excretion route	Time after administration				
	30 min	1 hr	3 hr	6 hr	12 hr
Urine	9.44	19.08	63.23	86.54	88.85
Feces	—	0.005	0.03	0.24	1.41

^{a)} Each value represents the mean of 3 rats.

total excretions were $94.2 \pm 0.6\%$ and $3.37 \pm 0.25\%$ of the total dose in 20 days, respectively. These values were approximately equal to the values of single administration, showing no influence of repeated dosing.

4. Determination of Urinary Metabolite

Since the major part of radioactivity was excreted to urine after the oral administration of ^{14}C -ICA, the identification and determination of metabolite in rat urine were studied by thin layer chromatography and isotope dilution method.

A small volume of filtered rat urine collected in 24 hours after single oral administration was applied to thin layer chromatography using 2 kind of developing solvents, dioxane/*n*-hexane=1/1 and pyridine/dimethylformamide/*n*-BuOH=1/1/8. For the control, the same procedure was conducted with the solutions of reference labeled compound dissolved in normal rat urine and water separately.

The chromatograms obtained by use of the former solvent were shown in Fig. 4. Each of these materials showed only one radioactive peak using any of developing solvents. The position of the peak of excreted urine on chromatogram was quite identical to that of the solution of ^{14}C -ICA in normal rat urine, though the R_f values of both of them were a little smaller than that of the aqueous solution of the labeled compound. This little difference was thought to be due to the influence of salts contained in urine, therefore, it was noted that any metabolite other than ICA was not found in urine.

Moreover, the urine of 6 rats collected in 12 hours from evening of 17 th day to morning of 18 th day of repeated oral administration of ^{14}C -ICA for 20 days was used for the verification of excreted ICA by isotope dilution method.

Fig. 4. Thin-Layer Chromatograms of Rat Urine and ^{14}C -Isocyanuric Acid Solution

A : ^{14}C -ICA solution in water, $R_f=0.31$.
 B : ^{14}C -ICA solution in normal rat urine, $R_f=0.28$.
 C : Urine of rat after ^{14}C -ICA administ., $R_f=0.28$.
 Solvent : dioxan/*n*-hexane=1/1.

The urine was made exactly 400 ml by addition of a small volume of water and 10.0 g of reference ICA was added and dissolved by heating and filtered. The solid ICA in the solution was collected by filtration after allowing to stand overnight. About 50 mg of the dry solid was weighed accurately and the specific activity was measured every time after the repeated recrystallization. As shown in Table VIII, the radioactive crystal had a constant specific activity throughout 5 times recrystallization.

The ratio of radioactivity in the solid to whole urine was calculated from the weight of added ICA (10.0 g), the specific activity of crystal and the radioactivity of whole urine measured beforehand, and it was about 99%. Therefore, the compound showing about 99% of urinary radioactivity was identified to be

TABLE VIII. Specific Activity of Urinary Excreted Substance Extracted with Isoncyanuric Acid Carrier^{a)}

Time of recrystallization	Specific activity (dpm/mg)
1	11548
2	11634
3	11785
4	11799
5	11766

a) Radioactivity in whole urine : 11824×10^4 dpm.
ICA added to whole urine : 10.0 g.

intact ICA.

Discussion

1. Distribution

After both single and repeated oral administration of ^{14}C -ICA, the radioactivity distributed rather evenly to many organs and tissues investigated, but among these organs and tissues, higher radioactive concentrations were found in blood, liver and kidney, showing their peaks at 20 min after dosing. Thereafter, the decrease of concentration advanced rapidly, and the biological half times, estimated from Fig. 1, of radioactivity in blood and these organs after single administration were 65–70 min.

Accompanying with blood and these organs, the concentration in other organs and tissues also showed the highest value at 30 min after dosing, and thereafter the continuous decrease of concentration, roughly proportionate to blood, was observed even after the termination of repeated oral administration. Considering these results, accumulation or persistence of the compound in any of the organs and tissues might not be expected.

The radioactivity absorbed to blood after oral administration was prominently distributed to serum, namely, the ratio of radioactivity in serum to that in red cells was 10/1 and it was not changed with time lapse. So, the

cumulative absorption of this compound to red cells might not occurred practically.

2. Absorption

Considering the results of distribution after oral dosing, the major part of ^{14}C -ICA might be absorbed very rapidly from stomach and upper part of small intestine, though the absorption after repeated administration was a little delayed, comparing with the case of single dosing (Table II and V). Although 1–3 % of dose was found in rinsed skin of applied area, percutaneous absorption of the compound to body was regarded as very scarce, detecting no radioactivity in blood and only less than 0.01 % of dose in total urine in 21 hours after application.

Regarding the results of animal experiment mentioned above, any injurious effect caused by percutaneous absorption of cyanurate in swimming pool may not be taken into consideration.

3. Excretion

It was observed that very small quantity of radioactivity was excreted to expiration after oral administration, and even in the case of intravenous injection only about 1.2 % of dosed radioactivity was excreted in 12 hours after dosing. The peak of excretion appeared at 4–6 hours after dosing of both routes, and this was somewhat delayed from the time showing peak of distribution to blood and lung.

Although the reason for this delay could be attributable partially to the time necessary for sampling the expired gas, some metabolic action was supposed to be expected. The expired radioactivity was presumed to be $^{14}\text{CO}_2$ from the condition of experiment, so the metabolic action for converting ICA to CO_2 was not noted practically after oral administration and was regarded as very minor

in the case of intravenous injection.

The biliary excretion of radioactivity after oral administration was also very rare and total excretion in 12 hours after dosing was less than 0.5 % of dose. So the entry of this compound into entero-hepatic circulation was regarded as inconsiderable. The strongest radioactivity was seen in the fraction collected from 30 min to 1 hour after dosing, but the real peak was supposed to be earlier, regarding the time for preserving eliminated bile in polyethylene tube cannulated to bile duct. This fact was in good agreement with the result of distribution to organs and tissues.

After single oral administration, the major part of ICA was excreted promptly to urine, namely, about 87 % of dose in 6 hours and 90 % in 12 hours were excreted. While only about 1.5 % of dose was excreted to feces in 12 hours after dosing. This finding was supported by the results of distribution (Table I and II), showing great decrease at 6 hours, almost entire disappearance of radioactivity at 12 hours after dosing from the organs including kidney and gastrointestinal tract. After repeated oral administration of the labeled compound, a quite similar result was obtained, therefore, no accumulating effect was acknowledged.

4. Metabolism and Biodegradation

The major part of radioactivity was excreted to urine after oral administration, and about 99 % of urinary radioactive substance was identified as intact ICA. Because the fecal and biliary excretions were in a few rate and the excretion to expiration was very scarce, it was thought that, ICA given orally might not undergo any metabolic action in the internal organs such as liver, and only a

very little probability of metabolic action, by which the compound was converted to CO₂, was expected. Nadkarni *et al.*⁷⁾ reported that ¹⁴C-triethylenimino-1,3,5-triazine (I) injected intraperitoneally to mouse was prominently excreted to urine within 24 hours and the principal radioactive metabolite was identified as cyanuric acid (II). Moreover the exhaled gas of mouse contained little radioactivity. Considering this result, the result of the present study was regarded as reasonable and ICA might be considered to be a very stable compound against the metabolic action.

It was acknowledged that ICA given orally did not decompose in rat body. However, as stated by Saldick,⁸⁾ the biodegradation of ICA in domestic effluent is of interest of environmental problem. According to the report, cyanuric acid is biodegraded readily under a variety of natural conditions such as activated sludge, sewage, soils, muds and river waters, particularly in systems of low dissolved-oxygen level. Therefore, for the further reference study on the biodegradation of ICA, the following experiment was conducted.

Each 10 ml of rat urine, collected in 24 hours after single oral administration of ¹⁴C-ICA, was taken in a wide mouth bottle of 250 ml, and 5 g of normal rat feces and 40 ml of water were added to mix. The bottles were allowed to stand, some of them were as it was (aerobic condition) and others were saturated with nitrogen gas (anaerobic condi-

7) M. V. Nadkarni, E. I. Goldenthal and P. K. Smith, *Cancer Research*, **14**, 559 (1954).

8) J. Saldick, *Appl. Microbiol.*, **28**, 1004 (1974).

tion), without shaking or agitation at room temperature.

At some time intervals, thereafter, the generated gas in the bottles were introduced and absorbed to toluene base scintillator solution containing ethanolamine, by passing air or nitrogen through the mixture in bottle. Then the radioactivities in scintillator were measured to survey the degradation rate of ICA. Only after 62 days, the radioactivity in the mixture was determined instead of the gas and the degradation rate was calculated, because of the difficulty of collecting the generated gas for long time.

The result was shown in Table IX.

TABLE IX. Degradation Rate of Excretory ^{14}C -Isocyanuric Acid Mixed with Rat Feces by Allowing to Stand (%)

Medium	Days of allowing to stand			
	1	3	7	62
Aerobic	0.04	0.26	25.89	91.29
Anaerobic	0.04	0.57	36.13	91.12

Under both aerobic and anaerobic conditions, remarkable degradation was found in 1 week, after a lag time of about half week, and after 62 days more than 90% of radioactive compound was decomposed. In 1 week, furthermore, the degradation rate under anaerobic condition was apparently superior to aerobic condition. These results were not changed by making the content of bottles acidic before passing air or nitrogen.

As described above, ICA excreted to rat urine in the intact form was degraded by contact with the mixture of urine and feces, probably by the action of enteric microorganism, under both of aerobic and anaerobic conditions. Considering the present result together with the result reported by Saldick, almost complete degradation of ICA in environment may be expected and the environmental contamination by ICA may not be regarded as a serious problem.

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