COMPARATIVE STUDIES ON THE FATE OF ¹⁴C-DIPYRIDAMOLE (RA8) AFTER SINGLE AND MULTIPLE ADMINISTRATION IN RATS

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Abstract.....1) Absorption, distribution and excretion of ¹⁴C-Dipyridamole (RA 8) were studied comparatively after the administration of single intravenous (5 mg/kg), single oral (10 mg/kg) and multiple oral (10 mg/kg, once a day for one week) dosages in rats.

2) The blood level of radioactivity after oral administration showed a monophasic slow elimination with a half-life ($t_{1/2}$) of about 10 hrs. The apparent absorption rate was not so high ($t_{1/2}$: 0.72 hr), therefore, it took about 3 hrs to reach the maximum level.

3) When the drug was applied intravenously, radioactivity in blood decreased tri-phasically, but the terminal phase, which appeared 12 hrs after the application, showed the same elimination rate as that of p. o. application, i. e. $t_{1/2}$: about 10 hrs.

4) The observed values of radioactivity during and after the multiple dosing regimen fitted well on the simulation curve derived from the results of a single administration.

5) Radioactivity was mainly distributed in the alimentary canal, liver and kidney, which were isolated after single or multiple oral administration. No radioactivity was found in CNS. Whole body autoradiogram obtained after intravenous or oral administration also supported the results of distribution as above.

6) No considerable accumulation of radioactivity was recognized in any tissues after the multiple dose in the rat.

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INTRODUCTION

Dipyridamole (RA 8), developed by Dr. Karl Thomae GmbH (Biberach/Riss, West Germany), is widely used clinically to improve ischemic heart disease. Absorption, metabolism, distribution and excretion of this drug had been investigated in rats (Beisenherz et al., 1960; Kunz et al., 1963; Mellinger and Bohorfoush, 1964; Mellinger and Bohorfoush, 1965; Kopitar, unpublished), rabbits (Beisenherz et al., 1960; Mellinger and Bohorfoush, 1964; Mellinger and Bohorfoush, 1965; Zak et al., 1963), dogs (Beisenherz et al., 1960; Zak et al., 1963; Kadatz and Beisenherz, 1965) and man (Beisenherz et al., 1960; Knipping et al., 1960; Beisenherz et al., 1965; Mellinger and Bohorfoush, 1966).

The present study aimed at testing the accumulation characteristics of RA 8 in tissues when ^{14}C -RA 8 was administered orally repeatedly to rats.

MATERIALS AND METHODS

1. Radiolabelled compound

^{14}C -RA 8 was synthesized in the radioisotope laboratory of Dr. Karl Thomae GmbH. The structure of RA 8 and the position of the radioisotope labelling are shown in Fig. 1. The specific activity of ^{14}C -RA 8 used was $7.3 \mu\text{Ci}/\text{mg}$ ($3.68 \text{ mCi}/\text{mmole}$). This exhibited a single peak of radioactivity on the TLC plate, for which acetone was used as a developing solvent.

Fig. 1. Chemical structure of RA 8 and the position of ^{14}C -labelling (*).

2. Animal

Male rats of the Sprague-Dawley strain weighing 180-210 g, aged 7 weeks, were used. During the experiments, they were kept under conditions of temperature $22 \pm 1^\circ\text{C}$ and of humidity $60 \pm 5\%$ in a metabolic cage, from which urine and feces were collected separately. Water and diet were given ad libitum.

3. Administration of drug

^{14}C -RA 8 crystal was dissolved with or without unlabelled RA 8 in solution, pH 2.4, containing 5 g of PEG 600 and 0.2 g of tartaric acid per 100 ml of water, to produce $7.3 \mu\text{Ci}/2 \text{ mg/ml}$ for intravenous administration, $7.3 \mu\text{Ci}/\text{mg/ml}$ for single oral administration and $4.9 \mu\text{Ci}/\text{mg/ml}$ for multiple oral administration. The drug solutions were administered to rats in a dose of 5 mg/kg via the tail vein, or in a dose of 10 mg/kg in the stomach with a tube.

4. Measurement of radioactivity in blood

Blood samples were taken from the orbital plexus at various intervals after administration, and were sampled in a volume of $50 \mu\text{l}$ put into liquid scintillation counting vials in pairs. After an addition of 0.3 ml of Protosol solution [Protosol (New England Nuclear Co., U. S. A.) 1 : Ethanol 2], the blood samples were solubilized by incubation at 55°C for 1 hr, and were decolorized by 0.3 ml of H_2O_2 ($>30\%$) under incubation again at 55°C for 30 min; and then 15 ml of Biofluor (New England Nuclear Co., U. S. A), liquid scintillator, and 0.5 ml of 0.5 N HCl were mixed in.

5. Measurement of radioactivity in tissues

Animals were killed by drawing of blood from the abdominal aorta, and various tissues were removed immediately, weighed and treated as follows according to the classification described below :

Group 1. Stomach, Small intestine, Large intestine.

Group 2. Liver, Kidney, Spleen, Testis, Lung, Heart, Brain.

Group 3. Hypophysis, Adrenal gland, Prostate, Epididymis, Thyroid gland, Sublingual gland.

Group 4. Diaphragm, Thymus, Parotid gland, Submandibular gland, Pancreas.

Group 5. Adipose tissue, Skeletal muscle.

To each tissue in Group 1 water was added (4 times volume of tissue weight), and this mixture was then homogenized with Ultra-Turrax (Janke & Kunkel Co., West Germany). The homogenate was sampled, about 200 mg being placed in a counting vial. Each tissue in Group 2 was mashed to a paste-like consistency with embryo-extractor (Ikemoto-Rika Co., Japan) and completely homogenized. The homogenate was sampled, about 150 mg being placed in a counting vial. Each whole tissue in Group 3 was put directly into a vial. Each tissue in Group 4 and 5 was minced with scissors, and parts of these samples were placed in the vial.

To each of these samples in the counting vials was added 1.3 ml of Protosol, the resultant mixtures were incubated at 55°C for 5 hrs so as to be dissolved completely, and then decolorized with 0.3 ml of H_2O_2 under incubation at 55°C for 1 hr. The procedure

hereafter was the same as in the case of blood. The proximal portion of the intestine just up to the caecum was regarded as the small intestine, and the following part of the intestine, including the caecum, was regarded as the large intestine.

6. Whole body autoradiography

Autoradiogram was made according to a method described by Ullberg (1954). Rats were sacrificed 15 min after intravenous administration or 3 hrs after oral administration of ^{14}C -RA 8. Slice preparations were placed in close contact with X ray film (Sakura-type N, Konishiroku Photo Ind. Co., Japan) for 6 weeks at 4°C.

7. Measurement of radioactivity excreted in urine and feces

Feces were mixed with a quantity of water 9 times their volume and homogenized with Ultra-Turrax. About 200 mg of the homogenate was sampled being placed in a vial and treated the same as the tissues.

Urine was sampled in a volume of 100 μl and put directly into a vial, to this was added 15 ml of Aquasol (New England Nuclear Co., U. S. A.), the liquid scintillator.

8. Measurement of radioactivity by LSC

A liquid scintillation spectrometer (Model ABAC SL40, Intertechnique Co., France) was used for the measurement of radioactivity. The resulting data were corrected for quenching by the method of external standard ratio technique. All the values of radioactivity were calculated as the quantity of RA 8 equivalent.

RESULTS

1. Levels of radioactivity in blood

1) Intravenous administration

Changes in the blood concentration of radioactivity after the intravenous administration of ^{14}C -RA 8 in a dose of 5 mg/kg to rats are shown in Fig. 2. The elimination process of radioactivity in blood was divided into three phases as follows :

$$C_{iv} = 2890e^{-2.613t} + 103e^{-0.369t} + 18.2e^{-0.0642t} \quad (\text{ng/ml}) \quad (1)$$

This equation gives the biological half-lives of the radioactivity in blood as 0.3, 1.9 and 10.8 hrs in each phase, respectively.

2) Single oral administration

Changes in the blood concentration of radioactivity after the oral administration of ^{14}C -RA 8 10 mg/kg to rats are shown in Fig. 2.

The concentration of radioactivity increased gradually and reached the maximum 3 hrs after administration and subsequently decreased slowly. The phase following the maximum showed a linear decrease in semi-logarithmic plot. It appeared that the elimination might be apparently fitted to a one compartment pharmacokinetic model, and resulted in the following expression obtained by the method of least squares :

Fate of dipyridamole

Fig. 2. Time course of the concentration of radioactivity in blood after intravenous and oral administration of ^{14}C -RA 8 (5 mg/kg i. v., 10 mg/kg p. o.) in rats. Each point represents the mean $\pm$ S. D. of 4 (i. v.) and 3 (p. o.) animals.

$$C_{po} = -115.7e^{-0.955t} + 115.7e^{-0.0681t} \quad (\text{ng/ml}) \quad (2)$$

The half-lives in each phase were calculated as 0.7 and 10.2 hrs.

3) Multiple oral administration

^{14}C -RA 8 was administered once a day for 7 days in a dose of 10 mg/kg to rats. The concentration of radioactivity was measured just before and 3 hrs after each administration for 6 days and at various times after the final administration on the 7th day. Results are shown in Fig. 3. The experimental values fitted well on the simulation curve which was derived from equation 2 for the time course after single oral administration. Upon multiple dosing the blood levels of the drug ($C_{(n)}$) during any dosing interval n are given by

$$C_{(n)} = A \left(\frac{1-e^{-nk\tau}}{1-e^{-k\tau}} e^{-kt} - \frac{1-e^{-nk_a\tau}}{1-e^{-k_a\tau}} e^{-k_a t} \right) \quad (\text{ng/ml}) \quad (3)$$

where $0 \leq t \leq \tau$. And values for A , k and k_a are described in equation 2. The results demonstrated little possibility of accumulation of RA 8 in the body of rats.

2. Distribution of radioactivity in tissues

1) Single oral administration

Fig. 3. Simulation curve in the multiple dosing regimen, once a day for one week. Calculation was based on parameters obtained from the curve after single oral administration of ^{14}C -RA 8. Each point represents the mean of experimental value $\pm$ S. D. of 6 animals.

Distribution of radioactivity in various tissues was investigated 3 hrs after administration, at this time the concentration of radioactivity in blood was assumed to reach its maximum, and 8 and 24 hrs after the administration of ^{14}C -RA 8 (Table 1). An extremely large amount of radioactivity was found in the stomach, small intestine or large intestine including their contents at 3 hrs. These values increased or decreased depending on the movement of the materials in G. I. tract in the course of time. In the stomach which was the place of drug application only a trace of radioactivity was recognized 24 hrs after. However, radioactivity still existed at a level of about 15% of the original dose in the small intestine and 60% in the large intestine 8 hrs after administration. Furthermore, radioactivity was present in the liver at high levels and hardly decreased in the course of time. Concentration of radioactivity in the kidney and adrenal gland was also found in the second highest. No radioactivity was observed in the brain.

2) Multiple oral administration

Table 2 shows the distribution of radioactivity 24, 48 and 72 hrs after multiple oral administration of ^{14}C -RA 8. Concentrations of radioactivity were highest in the liver, and second highest in the kidney and adrenal gland, a distribution was similar to that observed after single administration. The values at 24 hrs in the liver and kidney were higher than those at the same period after single administration. Generally, the values of distribution of radioactivity obtained after the multiple administration were higher and the elimination rates were lower than those after single administration, but there was not such a great difference, suggesting a low accumulation tendency.

3. Whole body autoradiography

1) Intravenous administration

Radioactivity was observed in the whole body of the rat except the brain and spinal cord 15 min after intravenous administration of ^{14}C -RA 8. The highest radioactivity was shown in the liver, duodenum, mucous in glandular stomach and urine in the bladder. The second highest radioactivity was in the kidney, adrenal cortex and mucous in distal intestine. The harderian gland, salivary gland and arterial wall also showed weak radioactivity (Fig. 4).

2) Oral administration

Fig. 5 shows the autoradiogram 3 hrs after oral administration. Very strong radioactivity was demonstrated in the G. I. tract including contents. That in the liver was also relatively higher. The kidney and adrenal cortex showed weak radioactivity.

4. Rate of excretion of radioactivity in urine and feces

1) Single oral administration

Urine and fecal excretion of radioactivity measured at 24 hrs intervals after single oral administration of ^{14}C -RA 8 are shown in Fig. 6. In both urine and feces, radioactivity was observed to be substantially excreted within 24 hrs. The urinary excretion rate was extremely low ; only 5 % of the dose by 96 hrs, in contrast with the fecal excretion, approximately 87% by the same period.

2) Multiple oral administration

As shown in Fig. 7, during multiple administration over a period of 7 days, radioactivity was excreted in urine and feces every day in proportion to the number of doses of ^{14}C -RA 8 administered. Mean values of excreted percentage of dose for 7 days were 87% in feces and 4% in urine, being almost similar to the values obtained in the experiment for single administration.

Fig. 4. Whole body autoradiograms showing the distribution of radioactivity 15 min after intravenous administration of ^{14}C -RA 8 in a dose of 5 mg/kg in rats.

Upper = Midline section, Lower = Left side section

Fig. 5. Whole body autoradiograms showing the distribution of radioactivity 3 hrs after oral administration of ^{14}C -RA 8 in a dose of 10 mg/kg in rats.

Upper = Midline section, Lower = Left side section

Fate of dipyridamole

Fig. 6. Cumulative urinary and fecal excretion of radioactivity after single oral administration of ^{14}C -RA 8 in a dose of 10 mg/kg in rats. Each point represents the mean $\pm$ S. D. of 6 animals.

Fig. 7. Cumulative urinary and fecal excretion of radioactivity during and after multiple oral administration of ^{14}C -RA 8 in a dose of 10 mg/kg, once a day for one week in rats, and cumulative dose administered for one week. Each point represents the mean $\pm$ S. D. of 12 (1-7th day), 9 (8th day), 6 (9th day) and 3 (10th day) animals.

DISCUSSION

Whether RA 8 is accumulated in animals was tested in rats. Blood level, distribution in various tissues, and rate of urinary and fecal excretion after the multiple oral administration of ^{14}C -RA 8, 10 mg/kg once a day for 7 days, were compared with those after a single administration of the drug.

When ^{14}C -RA 8 in a dose of 5 mg/kg was applied intravenously, the elimination pattern of radioactivity was different from that resulting from oral administration, and it was rather rapid. Namely, the pattern of elimination after an intravenous administration was tri-phasic and the biological half-lives were 0.3, 1.9 and 10.8 hrs in each phase respectively. On the contrary, the level of radioactivity after oral administration reached its maximum slowly at about 3 hrs, and then decreased seemingly mono-phasically. The value of the half-life of elimination was 10.2 hrs, showing a close similarity to that of the third phase in elimination, that appeared about 12 hrs after intravenous administration (Fig. 2).

Beisenherz et al. (1960) reported that RA 8 was well absorbed, which was evidenced by the fact that 55% of the dose had disappeared from the G. I. tract within 30 min after oral administration in rats. And at this time, 20% of the dose was found as glucuronide of RA 8 in the small intestine. Mellinger et al. (1965) observed that RA 8 could be detected in the serum of the portal vein within 30 sec after administration into the stomach, therefore, the absorption was very swift, but the rate of excretion in bile and of reabsorption from the intestine were high, so that the concentration of RA 8 in the portal vein was maintained at a high level for a long time. On the other hand, in the case of intravenous administration, no difference was observed between the concentration of RA 8 in the serum of the arterial system and that in the serum of the portal vein, and this fact was assumed to cause a decline of drug level nearly 10 times as rapid as that following oral administration. These findings suggest the lack of an established enterohepatic circulation after intravenous administration. The apparent lack of a second (or first) phase of elimination in the case of oral administration, though such a phase appeared after i. v. administration, might have been attributed to that the second phase were masked by such a large enterohepatic circulation.

The simulation curve for the multiple dosing regimen, which was calculated and illustrated on the basis on parameters for the expression of the time course following the single oral administration, showed that the plateau levels for both the maximum and the minimum concentration were achieved within 4-5 days after the beginning of dosing. The experimental values fitted the simulation curve very well. It was presumed from the results described above that RA 8 has almost no tendency to accumulate in the living body. Mellinger and Bohorfoush (1966) reported the same results, showing no accumulation of RA 8 in humans who were administered multiple doses of RA 8 (25-75 mg, three times a day for 2 weeks), and showing in addition that the average concentration of RA 8 in serum reached a plateau by the 3rd-4th day and

that the concentration changed widely within every 24 hr period. Beisenherz et al. (1960) also reported the same evidence in humans.

The liver and kidney showed the highest distribution of radioactivity after both the single and multiple dosing (Tables 1 and 2). The RA 8 radioactivity was excreted in bile

Table 1. Distribution of radioactivity 3, 8 and 24 hours after single oral administration of ^{14}C -RA 8 in a dose of 10 mg/kg in rats. The value show the mean of ng equivalent of RA 8/g wet weight of tissue $\pm$ S. D. of three animals.

Tissue	3 hr		8 hr		24 hr	
Liver	7,615.4 $\pm$ 1,511.7	(10.81 $\pm$ 0.63)	7,628.6 $\pm$ 841.8	(10.89 $\pm$ 0.47)	4,130.8 $\pm$ 1,079.5	(9.26 $\pm$ 1.00)
Kindney	618.3 $\pm$	50.8 (1.85 $\pm$ 0.09)	477.0 $\pm$ 105.3	(1.83 $\pm$ 0.05)	276.5 $\pm$	47.0 (1.60 $\pm$ 0.10)
Whole brain	24.3 $\pm$	26.2 (1.70 $\pm$ 0.09)	6.0 $\pm$	2.1 (1.71 $\pm$ 0.10)	1.8 $\pm$	0.9 (1.72 $\pm$ 0.07)
Testis	32.3 $\pm$	6.1 (2.17 $\pm$ 0.13)	13.7 $\pm$	0.5 (2.36 $\pm$ 0.05)	8.3 $\pm$	1.6 (2.12 $\pm$ 0.04)
Lung	214.9 $\pm$	41.5 (1.05 $\pm$ 0.07)	131.9 $\pm$	31.0 (1.04 $\pm$ 0.08)	42.9 $\pm$	12.5 (1.05 $\pm$ 0.09)
Pancreas	156.8 $\pm$	25.9 (0.757 $\pm$ 0.307)	87.2 $\pm$	14.1 (0.857 $\pm$ 0.03)	40.6 $\pm$	11.6 (0.812 $\pm$ 0.286)
Spleen	148.3 $\pm$	11.3 (0.624 $\pm$ 0.080)	52.0 $\pm$	1.1 (0.668 $\pm$ 0.066)	25.8 $\pm$	7.2 (0.873 $\pm$ 0.051)
Heart	142.4 $\pm$	36.7 (0.674 $\pm$ 0.054)	46.4 $\pm$	11.7 (0.696 $\pm$ 0.052)	18.3 $\pm$	1.8 (0.711 $\pm$ 0.010)
Prostate	76.5 $\pm$	11.7 (0.161 $\pm$ 0.028)	39.5 $\pm$	2.5 (0.154 $\pm$ 0.028)	22.1 $\pm$	9.0 (0.138 $\pm$ 0.004)
Epididymis	80.5 $\pm$	7.2 (0.293 $\pm$ 0.003)	38.5 $\pm$	6.8 (0.307 $\pm$ 0.020)	19.4 $\pm$	3.0 (0.289 $\pm$ 0.021)
Adrenal gland	856.1 $\pm$	169.3 (0.032 $\pm$ 0.009)	205.0 $\pm$ 163.6	(0.034 $\pm$ 0.003)	91.4 $\pm$	4.4 (0.039 $\pm$ 0.003)
Hypophysis	121.3 $\pm$	56.8 (0.012 $\pm$ 0.001)	73.8 $\pm$	55.0 (0.008 $\pm$ 0.004)	17.8 $\pm$	22.4 (0.009 $\pm$ 0.003)
Thyroid gland	167.8 $\pm$	65.3 (0.016 $\pm$ 0.003)	82.4 $\pm$	8.3 (0.016 $\pm$ 0.002)	79.6 $\pm$	18.2 (0.020 $\pm$ 0.003)
Thymus	88.0 $\pm$	17.5 (0.664 $\pm$ 0.105)	41.7 $\pm$	3.6 (0.681 $\pm$ 0.100)	22.7 $\pm$	4.5 (0.491 $\pm$ 0.061)
Parotid gland	131.8 $\pm$	20.7 (0.191 $\pm$ 0.045)	64.4 $\pm$	10.6 (0.309 $\pm$ 0.094)	50.8 $\pm$	6.2 (0.302 $\pm$ 0.132)
Sublingual gland	111.9 $\pm$	25.8 (0.079 $\pm$ 0.024)	50.2 $\pm$	11.9 (0.077 $\pm$ 0.010)	32.4 $\pm$	6.1 (0.060 $\pm$ 0.012)
Submandibular gland	143.8 $\pm$	26.0 (0.365 $\pm$ 0.030)	63.4 $\pm$	16.1 (0.364 $\pm$ 0.010)	26.0 $\pm$	3.5 (0.367 $\pm$ 0.047)
Diaphragm	158.4 $\pm$	52.8 (0.494 $\pm$ 0.105)	67.5 $\pm$	23.9 (0.454 $\pm$ 0.070)	19.6 $\pm$	3.1 (0.415 $\pm$ 0.117)
Adipose tissue	105.1 $\pm$	15.7	73.0 $\pm$	22.9	29.2 $\pm$	2.6
Skeletal muscle	49.0 $\pm$	14.8	22.5 $\pm$	2.9	11.4 $\pm$	2.8
Blood	*140.4 $\pm$	16.5	*43.4 $\pm$	10.8	*11.0 $\pm$	3.9
Stomach & contents	**310.1 $\pm$	279.2 (5.67 $\pm$ 1.16)	**52.8 $\pm$	73.1 (2.30 $\pm$ 0.96)	**0.2 $\pm$	0.0 (5.18 $\pm$ 1.76)
Small intestine & contents	**913.6 $\pm$	534.2 (10.42 $\pm$ 0.44)	**281.2 $\pm$ 122.8	(9.82 $\pm$ 1.31)	**10.9 $\pm$	3.5 (11.60 $\pm$ 0.67)
Large intestine & contents	**916.4 $\pm$	706.3 (9.06 $\pm$ 1.42)	**1,130.5 $\pm$ 577.6	(8.13 $\pm$ 1.62)	**92.1 $\pm$	60.0 (7.01 $\pm$ 0.63)

*ng RA 8 equivalent/ml of whole blood

** μg RA 8 equivalent in whole tissue

The value in parenthesis represents the wet weight of whole tissue.

at a high rate as glucuronide, and then this was converted to free form in the intestine and reabsorbed immediately, that is, the entero-hepatic circulation of radioactivity was repeated (Beisenherz et al., 1960; Zak et al., 1963; Beisenherz et al., 1965) so that radioactivity was assumed to be highly concentrated in the liver and kidney for a long time. The values of distribution in the various tissues obtained 24 hrs after the multiple

Table 2. Distribution of radioactivity 24, 48 and 72 hours after multiple oral administration of ^{14}C -RA 8 in a dose of 10 mg/kg, once a day for one week in rats. The value show the mean of ng equivalent of RA 8/g wet weight of tissue $\pm$ S. D. of three animals.

Tissue	24 hr		48 hr		72 hr	
Liver	9,463.9 $\pm$ 1,886.3	(10.19 $\pm$ 0.67)	3,963.9 $\pm$ 801.8	(12.43 $\pm$ 0.21)	2,050.9 $\pm$ 579.5	(10.40 $\pm$ 0.69)
Kidney	748.5 $\pm$	146.3 (1.88 $\pm$ 0.23)	613.4 $\pm$ 175.6	(2.00 $\pm$ 0.16)	335.0 $\pm$	48.6 (1.67 $\pm$ 0.13)
Whole brain	15.1 $\pm$	4.0 (1.67 $\pm$ 0.03)	11.7 $\pm$	7.2 (1.72 $\pm$ 0.09)	11.2 $\pm$	2.4 (1.78 $\pm$ 0.02)
Testis	45.4 $\pm$	16.8 (2.62 $\pm$ 0.13)	33.1 $\pm$	7.1 (2.78 $\pm$ 0.45)	21.6 $\pm$	2.4 (2.90 $\pm$ 0.21)
Lung	119.6 $\pm$	26.6 (0.997 $\pm$ 0.047)	92.6 $\pm$	8.1 (1.05 $\pm$ 0.19)	44.6 $\pm$	3.1 (1.09 $\pm$ 0.15)
Pancreas	111.2 $\pm$	36.2 (0.727 $\pm$ 0.212)	79.7 $\pm$	29.4 (0.839 $\pm$ 0.291)	65.5 $\pm$	10.7 (0.689 $\pm$ 0.126)
Spleen	102.1 $\pm$	12.1 (0.829 $\pm$ 0.165)	85.9 $\pm$	11.8 (0.793 $\pm$ 0.155)	61.1 $\pm$	6.6 (0.902 $\pm$ 0.169)
Heart	88.5 $\pm$	8.3 (0.739 $\pm$ 0.033)	77.4 $\pm$	11.4 (0.800 $\pm$ 0.045)	52.3 $\pm$	8.2 (0.729 $\pm$ 0.076)
Prostate	47.4 $\pm$	11.6 (0.208 $\pm$ 0.068)	55.2 $\pm$	34.2 (0.222 $\pm$ 0.058)	17.4 $\pm$	2.6 (0.177 $\pm$ 0.045)
Epididymis	67.7 $\pm$	5.3 (0.405 $\pm$ 0.029)	54.5 $\pm$	14.3 (0.415 $\pm$ 0.055)	30.4 $\pm$	2.9 (0.427 $\pm$ 0.020)
Adrenal gland	320.5 $\pm$	18.8 (0.047 $\pm$ 0.004)	393.2 $\pm$	73.6 (0.048 $\pm$ 0.004)	231.4 $\pm$	89.4 (0.052 $\pm$ 0.003)
Hypophysis	65.1 $\pm$	39.5 (0.010 $\pm$ 0.001)	55.8 $\pm$	9.6 (0.017 $\pm$ 0.000)	23.5 $\pm$	32.6 (0.020 $\pm$ 0.003)
Thyroid gland	153.5 $\pm$	99.3 (0.017 $\pm$ 0.002)	116.9 $\pm$	25.7 (0.017 $\pm$ 0.006)	27.0 $\pm$	10.8 (0.024 $\pm$ 0.002)
Thymus	74.2 $\pm$	5.2 (0.477 $\pm$ 0.060)	70.3 $\pm$	18.4 (0.487 $\pm$ 0.150)	38.1 $\pm$	22.0 (0.523 $\pm$ 0.153)
Parotid gland	137.0 $\pm$	36.8 (0.298 $\pm$ 0.001)	146.5 $\pm$ 112.2	(0.334 $\pm$ 0.055)	76.3 $\pm$	44.6 (0.346 $\pm$ 0.112)
Sublingual gland	76.4 $\pm$	18.6 (0.080 $\pm$ 0.022)	55.2 $\pm$	12.1 (0.087 $\pm$ 0.023)	25.2 $\pm$	4.1 (0.080 $\pm$ 0.010)
Submandibular gland	74.6 $\pm$	11.4 (0.379 $\pm$ 0.027)	50.9 $\pm$	7.8 (0.379 $\pm$ 0.037)	35.6 $\pm$	16.4 (0.402 $\pm$ 0.048)
Diaphragm	84.7 $\pm$	12.8 (0.491 $\pm$ 0.049)	92.0 $\pm$	35.3 (0.494 $\pm$ 0.027)	72.2 $\pm$	1.7 (0.588 $\pm$ 0.022)
Adipose tissue	113.7 $\pm$	20.5	161.9 $\pm$	77.1	73.5 $\pm$	25.5
Skeletal muscle	69.3 $\pm$	15.7	56.8 $\pm$	13.5	44.6 $\pm$	18.0
Blood	*22.9 $\pm$	3.6	*14.3 $\pm$	2.0	*8.4 $\pm$	0.6
Stomach & contents	**0.3 $\pm$	0.3 (4.63 $\pm$ 3.33)	**0.6 $\pm$	0.6 (6.06 $\pm$ 1.83)	**0.1 $\pm$	0.1 (5.55 $\pm$ 0.58)
Small intestine & contents	**1.9 $\pm$	0.0 (9.88 $\pm$ 0.66)	**0.5 $\pm$	0.0 (11.62 $\pm$ 0.48)	**0.5 $\pm$	0.3 (10.29 $\pm$ 0.63)
Large intestine & contents	**13.7 $\pm$	3.5 (6.99 $\pm$ 0.82)	**2.2 $\pm$	1.4 (10.29 $\pm$ 1.21)	**0.5 $\pm$	0.3 (7.29 $\pm$ 1.51)

*ng RA 8 equivalent/ml of whole blood

** μg RA 8 equivalent in whole tissue

The value in parenthesis represents the wet weight of whole tissue.

dosing were much higher than those obtained after the single dosing, except for in the central nervous system. Concentrated radioactivity in the liver and intestine was also demonstrated by the technique of whole body autoradiography which was carried out 3 hrs after oral administration (Fig. 5). Fig. 4, showing autoradiograms obtained after intravenous administration, also showed that radioactivity was excreted into the stomach and proximal portion of the intestine.

Kunz et al. (1963) reported that 15 min after intravenous administration in dose of 0.1-150 mg/kg to rats, RA 8 was distributed not only in the liver, lung and kidney but also in the heart muscle in high concentrations, that is, the concentration in the heart

was 5-16 times the value of blood. In our experiment, an autoradiogram 15 min after intravenous administration of ^{14}C -RA 8 showed a distinctly higher concentration of radioactivity in the heart muscle than in blood in the cardiac atria or ventricles.

The distribution of RA 8 had been also studied in other animals ; rabbits (Mellinger and Bohorfoush, 1964 ; Mellinger and Bohorfoush, 1965 ; Zak et al., 1963), mice (Kunz et al., 1963 ; Kopitar, unpublished) and guinea-pigs (Mellinger and Bohorfoush, 1965). For these experiments, the tissues were isolated after the dosing, or the ARG method was applied. All those studies showed both RA 8 or its glucuronide were distributed mainly in the entero-hepatic circulation system.

It was shown again by our experiment that the rate of urinary excretion of RA 8 was low, as had been reported (Beisenherz et al., 1960 ; Mellinger and Bohorfoush, 1965 ; Zak et al., 1963 ; Beisenherz et al., 1965 ; Mellinger and Bohorfoush, 1966) ; nevertheless absorption was well established (Beisenherz et al., 1960 ; Mellinger and Bohorfoush, 1965 ; Beisenherz et al., 1965), only 5.12% of the dose appeared in the urine within 96 hrs after the single oral administration. Results were similar for the multiple dosing ; that is, urinary excretion averaged about 4% over 7 days. Beisenherz et al. (1960) observed that the biliary excretion rate was 95% of dose within 5 hrs after intravenous administration of RA 8, and that 77% was excreted with bile within 6 hrs after intraduodenum application. Thus RA 8 is substantially excreted via bile into the intestine.

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