STUDIES ON METABOLISM OF TRIPAMIDE

III. METABOLIC FATE OF $^{14}$C-TRIPAMIDE
IN RATS AND RABBITS

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Abstract — The metabolic fate of a new antihypertensive agent, tripamide (N-(4-aza-endo-tricyclo[5.2.1,0.2,6]-decan-4-yl)-4-chloro-3-sulfamoylbenzamide), in rats and rabbits was studied using its $^{14}$C-labeled compound. The blood level of radioactivity in both species reached the maximum at 1 hr after oral administration, indicating the rapid absorption of the drug from the gastrointestinal tract. Following either oral or intravenous administration, a high concentration was observed in the liver of rats, but it was found in the kidney of rabbits. The major metabolite was 4-chloro-3-sulfamoylbenzoic acid in tissues, urine and feces; and in both species, the unchanged drug was only detected in the kidney. The pathway of excretion of radioactivity was via urine and feces in case of rats; but in rabbits, it was predominantly excreted via urine, although significant quantities of radioactivity were excreted with feces. The radioactivity excreted in feces was attributable to that which was excreted in bile, indicating the absorption of almost all the tripamide administered in both species. During the period of repeated dosing, though blood levels increased gradually and became about 1.5 times as high as that in the single dosing, radioactivity did not accumulate in most tissues and organs.

Tripamide, N-(4-aza-endo-tricyclo[5.2.1.0.2,6]-decan-4-yl)-4-chloro-3-sulfamoylbenzamide, has been developed as a non-thiazide compound with a tricyclocdecane ring and is a new antihypertensive agent with excellent pharmacological activity (1, 2), comparing favorably with the currently known thiazide drug.

Metabolic studies show that the compound is rapidly metabolized and forms five metabolites (3). The main metabolic pathways of tripamide were the hydroxylation at the tricyclocdecane ring and the hydrolysis of the amide bond. The hydroxylation reaction was catalyzed by monooxygenase, and the hydrolysis involved the liver arylamidase (4). Since species differences in the activities of monooxygenase and arylamidase were reported (5, 6), species differences in the metabolism of tripamide may also be observed in vivo and in vitro. We report here some findings on the absorption, distribution, metabolism and excretion of tripamide in rats and rabbits.

MATERIALS AND METHODS

Chemicals: $^{14}$C-Carbonyl-tripamide (Fig. 1) was prepared in our laboratory (7). $^{14}$C-Carbonyl-4-chloro-3-sulfamoylbenzoic acid ($^{14}$C-CSBA) which was obtained as the
intermediate product during the synthesis of \(^{14}C\text{-tripamide}\) was used in some of the metabolic studies. The radiochemical purities of \(^{14}C\text{-tripamide}\) and \(^{14}C\text{-CSBA}\) were proven to be more than 95% by TLC (Kieselgel GF\textsubscript{124}, Merck, Germany). \(^{14}C\text{-tripamide}\) having a specific radioactivity of 18.7 \(\mu\text{Ci}/\text{mg}\) was used for metabolism in rats and that of 11.6 \(\mu\text{Ci}/\text{mg}\) was used for metabolism in rabbits. \(^{14}C\text{-CSBA}\) had a specific radioactivity of 31.7 \(\mu\text{Ci}/\text{mg}\).

Animals: Male Wistar rats weighing 200–250 g and male New Zealand White rabbits weighing 2.6–3.1 kg were maintained on a pellet diet from Nihon Crea Co., Ltd (Japan). Rats were fasted overnight prior to use, but the rabbits were not. Tap water was given ad libitum.

Administration: \(^{14}C\text{-tripamide}\) was given orally to rats and rabbits by gastric intubation as a solution of 1.25% hydrochloric acid, and the solution for intravenous injection was adjusted to pH 10 by adding solid sodium hydroxide. \(^{14}C\text{-tripamide}\) was dissolved to produce a 1 mg/ml solution.

Determination of radioactivity: Radioactivity of each sample was measured by a Model LSC-653 liquid scintillation counter (Aloka, Japan). The quenching was corrected by an automatic external standard method. The composition of the scintillation liquid was composed of the following: dioxane (3 l), toluene (0.6 l) and ethyl cellosolve (0.4 l) plus naphthalene (400 g), PPO (16 g) and dimethyl POPOP (0.8 g).

Blood level of radioactivity: Fifty \(\mu\text{l}\) samples of blood from rats were taken from the tail vein, and 0.1 ml samples of blood from rabbits were taken from the ear vein. Each blood sample was plotted on filter paper, dried under air, and combusted to \(^{14}CO_2\) in the Tri-Cab Sample Oxidizer, Model 305 (Packard Instrument, U.S.A.) for counting radioactivity.

Tissue distribution of radioactivity: The animals were killed by decapitation at various time intervals after administration of the drug. The tissues of interest were immediately excised and washed with 0.9% NaCl and blotted on a filter paper. Approximately 200 mg of each fresh tissue was dried and subjected to combustion.

Urinary, fecal and biliary excretion of radioactivity: Each animal was individually housed in a metabolic cage, and urine and feces were collected. Cannulation of the common bile duct of rats was carried out under light ether anaesthesia. Twenty minutes after complete recovery from ether anaesthesia, the cannulated rats were given \(^{14}C\text{-tripamide}\) and were individually placed in restraining cages. The bile or urine was made up to a constant volume with water, an aliquot (0.1 ml) was placed in vials containing 15 ml of a dioxane scintillator, and the radioactivity was determined. Radioactivity of feces was determined after combustion using approx. 5–50 mg of dried feces.

Determination of metabolites: Animals were killed by decapitation, and the liver and kidney removed immediately and homogenized with a Teflon-glass homogenizer in 3 vol. of ice-cold water. The homogenate was freeze-dried and then refluxed with 50 ml of methanol for 4 hr at 80°C. The methanol extract was chromatographed on a Sephadex LH20 column to separate out the lipid component, and the eluate was evaporated to dryness in vacuo and analyzed by TLC. The blood sample was separated on an Amberlite XAD-4 column after it was hemolyzed with 15 vol. of water. Urine and bile were each also
directly applied on an Amberlite XAD-4 column. The metabolites were eluted from the column with distilled water, methanol-water (40:60, v/v), and methanol, in this order. Each eluate was evaporated to dryness in vacuo and analyzed by TLC. The dried feces (approx. 0.1 g) was refluxed with methanol for 4 hr at 80°C, and the methanol extracts used for the TLC assay.

**TLC assay:** The metabolites of the extracts were separated by TLC on silica gel containing a fluorescent indicator (Kieselgel GF254, 0.25 mm thickness, Merck, Germany) with two solvent systems used for development: 28% aqueous ammonia-water-n-butanol (1:9:50, v/v) and benzeneacetone (1:1, v/v). The radioactive spots were detected by autoradiography of the TLC plate mounted with X-ray film (Fuji Photo Film Co., Ltd. Japan). The spots corresponding to each metabolite on the chromatogram were scraped into an individual counting vial, and the radioactivity was determined.

**RESULTS**

**Blood level:** 14C-Tripamide was administered orally to rats or rabbits in a dose of 5 mg/kg. Figure 2 shows the time course of blood levels of radioactivity in rats and rabbits. Blood levels of radioactivity reach a maximum of 4.96 μg tripamide equiv./ml at 1 hr after oral administration to rats. The radioactivity disappeared from the blood with biphasic time curves having half-lives of 2.6 hr and 12.8 hr (Fig. 2). A peak was reached 1 hr after administration to rabbits, and the maximum concentration was 2.88 μg tripamide equiv./ml. The half-lives were 0.73 hr and 5.31 hr. (Fig. 2) The half-lives after intravenous injection were 0.03 hr and 2.63 hr in rats (0.6 mg/kg), but 0.06 hr and 1.51 hr in rabbits (0.3 mg/kg) (Fig. 3). Thus, the half-lives were faster in the rabbits than in the rats.

**Tissue distribution:** After oral administration of 14C-tripamide (5 mg/kg) to rats, the tissue distribution of radioactivity was examined...
Table 1. Tissue distribution of radioactivity in rats after oral administration of 14C tripamide (5 mg/kg)

| Tissues      | Time (hr) after administration | (μg tripamide equiv./g or ml) |
|--------------|---------------------------------|------------------------------|
|              | 2                               | 8                            | 96                            | 7 days |
| Brain        | 0.10±0.02                       | 0.07±0.02                    | 0                             | 0      |
| Testis       | 0.34±0.04                       | 0.14±0.01                    | 0                             | 0      |
| Nerve        | 0.18±0.02                       | 0.07±0.01                    | 0.01±0.00                     | 0      |
| Fat          | 0.27±0.04                       | 0.07±0.00                    | 0.02±0.00                     | 0      |
| Muscle       | 0.51±0.06                       | 0.17±0.01                    | 0.01±0.00                     | 0      |
| Pancreas     | 0.73±0.08                       | 0.71±0.06                    | 0.01±0.00                     | 0      |
| Heart        | 1.07±0.10                       | 0.41±0.02                    | 0.02±0.00                     | 0      |
| Spleen       | 1.34±0.04                       | 0.63±0.04                    | 0.06±0.01                     | 0.02±0.00 |
| Adrenal      | 1.43±0.20                       | 0.31±0.06                    | 0.01±0.00                     | 0      |
| Blood        | 4.11±0.19                       | 2.94±0.40                    | 0.14±0.01                     | 0      |
| Lung         | 1.25±0.09                       | 1.06±0.13                    | 0.03±0.00                     | 0.01±0.01 |
| Kidney       | 8.30±0.19                       | 2.80±0.07                    | 0.18±0.06                     | 0.04±0.02 |
| Liver        | 11.41±0.85                      | 3.59±0.33                    | 0.10±0.02                     | 0.05±0.01 |
| Intestine    | 3.45±0.94                       | 4.23±1.96                    | 0.10±0.00                     | 0      |
| Stomach      | 2.82±0.26                       | 0.85±0.02                    | 0.03±0.01                     | 0      |

Values represent the means±S.E. of three animals.

The highest radioactivity was found in the liver, followed by the kidney. In the other tissues, the radioactivity was lower than that in the blood; and there was almost no distribution of radioactivity in the brain, testis, nerves, fats and muscles. At 7 days after administration, almost no radioactivity could be observed in the body (0.05% of the dose administered). The results described above coincided with the results on the whole-body autoradiography of rats (8). After intravenous injection to rats, the tissue distributions of radioactivity were similar to those obtained after oral administration. No differences in the routes of administration could be observed.

The tissue distribution of radioactivity in rabbits was also examined after 14C-tripamide (5 mg/kg) was administered orally (Table 2). The highest radioactivity was found in the kidney, followed by the liver. The radioactivities in the other tissues were lower than that in the blood, and the lowest distributions of radioactivity were in the brain, nerves, fat and muscles. Radioactivity in the tissues 7 days after administration was 0.3% of the administered dose, and radioactivity had disappeared from almost all of the tissues.

Metabolism: Table 3 shows the metabolites of the liver and kidneys in rats (2 hr) and rabbits (1 hr) after oral administration of 14C-tripamide (5 mg/kg). A comparison of the metabolites in the liver and kidneys of both species shows that the major metabolite was 4-chloro-3-sulfamoylbenzoic acid (CSBA) in both tissues. The amount of the metabolite 4-chloro-3-sulfamoylbenzamide (CSBM) in rat liver was more than that in the rabbit; and in the kidney, it was less than that in the rabbit. The metabolite 4-chloro-3-sulfamoylbenzoic acid (N'-acetyl) hydrazide (CSBH) was not detected in the liver and kidney of rabbits. In both species, the unchanged drug was 9.6% (rats) or 3.4% (rabbits) of the metabolites in the kidneys, which are target organs of tripamide.
### Table 2. Tissue distribution of radioactivity in rabbits after oral administration of [14C] tripamide (5 mg/kg)

| Tissues   | Time (hr) after administration | 1            | 3            | 7 days     |
|-----------|---------------------------------|--------------|--------------|------------|
|           |                                 | (μg tripamide equiv./g or ml) |              |            |
| Brain     |                                 | 0.13±0.02    | 0.10±0.01    | 0.03±0.01  |
| Testis    |                                 | 0.32±0.10    | 0.32±0.05    | 0.02±0.00  |
| Nerve     |                                 | 0.13±0.06    | 0.43±0.02    | 0          |
| Fat       |                                 | 0.25±0.07    | 0.25±0.01    | 0.10±0.02  |
| Muscle    |                                 | 0.22±0.05    | 0.27±0.04    | 0          |
| Thymus    |                                 | 0.45±0.15    | 0.36±0.07    | 0.01±0.00  |
| Pancreas  |                                 | 0.85±0.32    | 0.50±0.04    | 0.03±0.01  |
| Heart     |                                 | 0.96±0.28    | 0.81±0.06    | 0.04±0.01  |
| Spleen    |                                 | 0.70±0.25    | 0.48±0.06    | 0.01±0.01  |
| Adrenal   |                                 | 1.07±0.42    | 0.64±0.16    | 0.01±0.00  |
| Blood     |                                 | 2.88±0.36    | 1.31±0.21    | 0          |
| Lung      |                                 | 1.48±0.57    | 1.12±0.17    | 0.01±0.01  |
| Kidney    |                                 | 20.87±8.01   | 10.13±2.46   | 0.11±0.01  |
| Liver     |                                 | 4.84±1.54    | 5.19±0.17    | 0.03±0.01  |
| Bile-cyst |                                 | 7.36±0.64    | 7.65±1.32    | 0.03±0.02  |
| Bile      |                                 | 0.06±0.01*   | 1.31±0.21*   | 0          |
| Intestine |                                 | 3.56±0.79    | 5.33±0.75    | 0.12±0.02  |
| Stomach   |                                 | 23.50±3.67   | 5.17±2.18    | 0.02±0.01  |

*Represents % of dose. Values represent the means±S.E. of three animals.

### Table 3. Concentration of tripamide and its metabolites in blood, liver and kidney after oral administration of [14C]tripamide (5 mg/kg) to rats and rabbits

| Metabolites     | Rat       | Rabbit     |
|-----------------|-----------|------------|
|                 | Liver     | Kidney     | Blood      | Liver     | Kidney     |
| Tripamime       | n.d.      | 9.6        | n.d.       | n.d.      | 3.4        |
| 8-Hydroxy-tripamime | 3.5      | 8.7        | n.d.       | n.d.      | 6.5        |
| CSBM            | 12.8      | 6.2        | 19.1       | 6.2       | 22.8       |
| CSBH            | 14.9      | 11.9       | 2.4        | n.d.      | n.d.       |
| CSBA            | 25.9      | 22.4       | 53.1       | 46.1      | 44.9       |
| Unknown metabolites | 42.9   | 41.2       | 27.9       | 47.4      | 22.4       |

The values represent % of metabolites in tissues 2 hr and 1 hr after oral administration of [14C]tripamide to rats and rabbits, respectively. n.d.: Not detectable. The abbreviations CSBM, CSBH and CSBA designate 4-chloro-3-sulfamoylbenzamide, 4-chloro-3-sulfamoylbenzoic acid-(N'-acetyl)hydrazide and 4-chloro-3-sulfamoylbenzoic acid, respectively.

Table 4 shows the metabolites in the bile, urine and feces. The major metabolite in the bile of rats was CSBA. The amounts of CSBA excreted in the urine and feces were compared in both species. In the rats, 10.1% was excreted in the urine and 5.6% in the feces. In the rabbits, however, 40.2% was excreted in the urine and 0.5% in the feces. Furthermore, the amounts of hydroxylated tripamide excreted in the urine and feces were compared in the rats and rabbits. In the rats, 2.9% of the dose was excreted in the urine, and 10.2%...
was excreted in the feces. In the rabbits, 2.6% of the dose was excreted in the urine, which was approx. the same as in the rats. However, the amount excreted in the feces of the rabbits was only 2.0%, which was about one-fifth of that in the rats. Amounts of the metabolites CSBM and CSBA which were excreted in rabbit urine were more than those in rat urine; and the urinary excretion of the metabolite CSBH in the rabbit was less than in the rat.

**Urinary and fecal excretion:** After $^{14}$C-tripamide (5 mg/kg) was administered orally to rats, 41.6% of the administered dose was excreted in the urine, and 47.0% was excreted in the feces during 7 days (Table 5). Approximately 90% of the dose was excreted in the urine and feces within the first 24 hr. Similar results were obtained after $^{14}$C-tripamide (5 mg/kg) was injected intravenously to rats.

After $^{14}$C-tripamide (5 mg/kg) was administered orally to rabbits, 80.1% of the dose was recovered in the urine during 7 days, and 95.5% was excreted within the first 24 hr, while 24.2% of the dose was recovered in the feces during 7 days (Table 5).

After $^{14}$C-tripamide (0.3 mg/kg) was

| Metabolites          | Rat (Bile) | Rat (Urine) | Rat (Feces) | Rabbit (Urine) | Rabbit (Feces) |
|----------------------|------------|-------------|-------------|----------------|----------------|
|                      | (% of dose)| (% of dose) | (% of dose) | (% of dose)     | (% of dose)     |
| Tripamide            | n.d.       | 0.2         | n.d.        | 0.2            | 0.4            |
| 8-Hydroxy-tripamide  | 0.1        | 2.5         | 5.0         | 1.3            | 1.1            |
| 3-Hydroxy-tripamide  | 0.7        | 0.4         | 5.2         | 1.3            | 0.9            |
| CSBM                 | 0.4        | 2.8         | 4.9         | 11.7           | 1.2            |
| CSBH                 | 0.6        | 4.8         | 2.4         | 0.9            | n.d.           |
| CSBA                 | 8.6        | 10.1        | 5.6         | 40.2           | 0.5            |
| Unknown metabolites  | 33.4       | 11.8        | 30.4        | 20.8           | 4.5            |

The values represent the means of three animals. n.d.: Not detectable. The abbreviations CSBM, CSBH and CSBA designate 4-chloro-3-sulfamoylbenzamide, 4-chloro-3-sulfamoylbenzoic acid-(N'-acetyl)hydrazide and 4-chloro-3-sulfamoyl-benzoic acid, respectively. The urinary and fecal excretion was tested in animals which were not cannulated to bile ducts.

| Days after administration | Rat (Urine) | Rat (Feces) | Rabbit (Urine) | Rabbit (Feces) |
|---------------------------|-------------|-------------|----------------|----------------|
|                           | (% of dose) | (% of dose) | (% of dose)     | (% of dose)     |
| 1                         | 38.5        | 44.3        | 76.4           | 8.6            |
| 2                         | 2.0         | 2.1         | 2.1            | 7.6            |
| 3                         | 0.5         | 0.3         | 0.8            | 4.0            |
| 4                         | 0.3         | 0.1         | 0.4            | 1.5            |
| 5                         | 0.2         | 0.1         | 0.2            | 1.2            |
| 6                         | 0.1         | 0.1         | 0.1            | 0.4            |
| 7                         | 0.0         | 0.0         | 0.1            | 0.9            |
| Total                     | 41.6        | 47.0        | 80.1           | 24.2           |

The values represent the means of three animals.
administered intravenously to rabbits, 63.7% of the dose was excreted in the urine within the first 48 hr, and 15.1% was excreted in the feces within this period. Since it is believed that the amount excreted into the feces were via the bile, it is assumed that the biliary excretion in rabbits is less than that in rats.

Biliary excretion: After 14C-tripamide (5 mg/kg) was administered orally to rats, biliary excretion began at 30 min after administration, and the cumulative excretion was 44.5% of the dose. After intravenous injection, biliary excretion was also 46.3% of the dose (Table 6).

Repeated administration: In rats, blood levels at 2 hr after oral administration increased gradually in 7-day repeated administrations of 14C-tripamide (4.5 mg/kg); during this period, the blood level of the 3rd day was about 1.5 times that of the first day (Fig. 4). At the end of the 7-day dosing, the blood level declined with a half-life of 32.8±1.9 hr posterior to 24 hr after the multiple dose, as was seen at 31.2±2.5 hr posterior to 24 hr after the single dose. On the 3rd day of 7-day repetitive dosing, the cumulative excretion of the day was 53.1±3.5% in feces and

**Table 6. Biliary excretion of radioactivity after oral and intravenous administration of [14C] tripamide (5 mg/kg) to rats**

| Time (hr) after administration | Oral (%) of dose | Intravenous (%) of dose |
|-------------------------------|-----------------|------------------------|
| 0.33                          | 0.5             | 0.5                    |
| 0.5                           | 0.1             | 3.1                    |
| 0.67                          | 1.0             | 3.8                    |
| 1                             | 2.8             | 7.9                    |
| 2                             | 3.0             | 6.2                    |
| 3                             | 3.2             | 3.7                    |
| 4                             | 2.7             | 7.8                    |
| 5                             | 3.8             | 9.2                    |
| 6                             | 3.8             | 9.2                    |
| 7                             | 3.8             | 9.2                    |
| 8                             | 2.4             | 4.0                    |
| 10                            | 3.5             | 9.2                    |
| 12                            | 16.3            | 9.2                    |
| 24                            |                 |                        |
| 48                            | 2.3             |                        |
| 72                            | 0.5             |                        |
| Total                         | 44.5            | 46.2                   |

The values represent the means±S.E. of three animals.
37.8±4.6% in urine, indicating almost the same pattern as seen with the single dosing (Fig. 5). At 10 hr after repeated administration, radioactivities in the kidneys, liver, and blood were higher than the other tissues and organs as shown in the study with a single dose, except for the finding that the distribution of radioactivity in the kidneys was higher than that in the liver at 10 hr after the 7-day repeated dosing. Repeated dosing produced no marked increase in radioactivity in organs and tissues as compared with the single dosing. However, the rate of disappearance of radioactivity in the spleen following the repeated dosing was slower than that of the single dose, and the level of radioactivity in the spleen 96 hr after the repeated dosing was a few times greater than that at 96 hr after the single dose (Table 1). Remaining radioactivities in tissues at 7 days after the 7-day repeated dosing was 0.16% of the administered dose.

**DISCUSSION**

In rats and rabbits, the blood levels of radioactivity rose quickly following oral administration of 14C-tripamide. This finding indicates that the drug is rapidly absorbed from the gastrointestinal tracts of both species. The disappearance of radioactivity in rabbit blood is more rapid than in rats when 14C-tripamide was administered orally or intravenously. In both species, the major metabolite in the blood was 4-chloro-3-sulfamoylbenzoic acid (CSBA). When 14C-CSBA was injected intravenously to rats (0.52 mg/kg) or rabbits (0.24 mg/kg), the half life was 65 min in rats and 1.7 min and 49.3 min in rabbits. Furthermore, the radioactivity recovered in the urine within the first 8 hr was about 20% of the administered dose in rats and about 100% in rabbits. The urinary recovery in rabbits was about 5 times greater than that in rats. Thus, it is suggested that the differences in the half life of radioactivity in the blood of both species after oral administration of 14C-tripamide might be due to differences in the excretion rate and pathway of CSBA.

A comparison of the excretion rates of the metabolites in the bile and urine shows that the excretion rate of the hydroxylated metabolites into bile is 2–3 times faster (3), which supports that in both species the amounts of the hydroxylated metabolites excreted in the feces are greater than that excreted in the urine as compared with other metabolites (See Table 4). The finding that the amounts of the hydroxylated metabolites excreted in the feces of rats were more than that of rabbits could be explained by the results that the biliary excretion of metabolites in the rabbits is lower than in the rats.

The species differences observed in the tripamide concentration of liver and kidney and in metabolic and excretion patterns suggests that the metabolic pathway of the drug may vary with animal species. An interesting finding in connection with the pharmacological activity of tripamide is that in both species, the unchanged drug was only found in the kidney, which are one of the target organs of tripamide.

Since the results obtained indicating that the hydrolysis of tripamide in tissues occurs rapidly and extremely in both species, the distribution and excretion of radioactivity after administration of 14C-tripamide are suggested to be attributable to that of CSBA. Baldwin et al. (9) reported that when 14C-iso-tripamide which was labeled with 14C at positions 3 and 5 of the tricyclodecane ring was administered orally to dogs, the radioactivity in liver and kidney also disappeared slowly as compared with the results obtained from 14C-carbonyl-tripamide.

Since biliary excretion is quite similar to fecal excretion, the radioactivity excreted in feces after oral administration of 14C-tripamide is caused via biliary excretion. In
addition, the radioactivity disappears rapidly from the blood and the liver, suggesting that there is little enterohepatic circulation of the metabolites and that in both rabbits and rats, almost all of the tripamide administered is excreted rapidly.

During the period of repeated dosing, though blood levels increased gradually and became about 1.5 times as high as that in the single dosing, the radioactivity in most tissues and organs did not increase greatly with repeated dosing and was almost the same as in the single dosing. A slight tendency for radioactivity retention in the spleen was observed in the single dosing, and this phenomenon became conspicuous in the repeated dosing study. This appeared to be derived from the radioactivity retained in the blood cells. It is probable that the phagocytotic destruction or lysis of blood cells by the splenic macrophages resulted in the redistribution of the radioactivity in the spleen, which will eventually be carried into the liver or kidneys for disposal. However, the finding that the biological half life of radioactivity in blood (32.8±1.9 hr) was shorter than the life span of the blood cells in rats (about 12 days) suggests that the affinity to the blood cells is not so strong as to remain inseparable until the cells are destroyed.

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