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# The Effect of Sacrificing Method on the Tissue Concentration of Exogeneous Creatinine in Rats

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The effect of sacrificing method on the apparent tissue concentration of creatinine, which is affected by the blood content of the tissue, was evaluated following the intravenous administration of creatinine to rats. Two methods were compared: the freezing method and the decapitation technique.

At 5 min following the administration, the creatinine concentrations in the brain and myocardium of the decapitated rats were significantly ($p < 0.05$) lower than those of the frozen rats. This might result from the difference of blood contents in these tissues between the two groups of rats. Thus, the experimental method for collecting tissues can affect the brain and myocardium concentrations of [$^{14}$C]creatinine administered to rats.

**Keywords**——creatinine; tissue concentration; sacrificing method; freezing; decapitation; tissue blood content

The concentrations of a drug in various tissues are usually determined to examine the distribution of the drug in experimental animals. However, different methods for sacrificing the experimental animals are sometimes used, and the blood contents in some tissues may possibly vary with the sacrificing methods. Therefore, when the difference between blood and tissue concentrations is large, the sacrificing method itself may affect the apparent tissue concentration.

It this paper, creatinine, which is a water-soluble chemical with low molecular weight, and is quantitatively excreted into the urine in unchanged form following *i.v.* administration,[1] was selected as a model chemical to clarify the effect of sacrificing method on the tissue drug concentration. The tissue concentrations were compared between rats frozen with dry ice–acetone and decapitated rats sacrificed at 5 and 60 min following *i.v.* administration of [$^{14}$C]creatinine.

## Materials and Methods

**Chemicals**——[carbonyl-$^{14}$C]Creatinine hydrochloride (specific activity, 16.0 mCi/mmol) was purchased from Amersham Corp., Amersham, England. The radiochemical purity was greater than 98%. All other chemicals were of analytical grade and were used without further purification.

**Animals**——Male Wistar rats were purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan, and were used when they weighed about 200 g. All rats were chronically cannulated into the left external jugular vein with silicone polymer tubing (i.d. 1.0 mm, o.d. 1.5 mm; Dow Corning, Tokyo, Japan) by the method of Upton.[2]

**Tissue Concentration Following *i.v.* Administration of [$^{14}$C]Creatinine**——Rats were given 10 $\mu$Ci/kg of [$^{14}$C]creatinine (0.70 mg/kg as creatinine) into the external jugular vein through the cannula, and sacrificed at 5 and 60 min following administration by soaking them in dry ice–acetone (−78 °C) under light anesthesia with ether or by decapitation with a guillotine (accompanied by extensive bleeding). About 50 mg of each tissue, *i.e.* heart blood,

brain, myocardium, liver, lung, muscle, thymus, spleen, testis and fat, was taken into a scintillation vial. The sampling position within each tissue and the method were the same after sacrifice by both methods. When myocardium was taken, the blood in the heart was removed with filter papers. Each sample was dissolved in 1 ml of tissue solubilizer (Soluene-350, Packard Instrument Co., Downers Grove, Ill., U.S.A.) and then decolorized with 50 $\mu$l of hydrogen peroxide.

**Radioactivity Measurement**——The radioactivity of each sample was determined in a Mark II liquid scintillation spectrometer (Nuclear-Chicago Corporation, Des Plaines, Ill., U.S.A.) with 10 ml of liquid scintillator (2,5-diphenyloxazole (PPO) 5 g, 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP) 0.3 g, toluene 700 ml, Triton X-100 300 ml). The counting efficiencies were automatically determined by the $^{133}$Ba external standard ratio method and cpm was converted to dpm.

## Results and Discussion

Creatinine is not metabolized in rats[1a] (though the microflora in the gastrointestinal tract can metabolize creatinine[1b]), and is quantitatively excreted into the urine following *i.v.* administration to rats.[1] Therefore, all of the radioactivity in blood and tissues following *i.v.* administration of [$^{14}$C]creatinine was regarded as originating from [$^{14}$C]creatinine.

Figure 1 shows the blood and tissue concentrations in rats sacrificed by freezing or decapitation at 5 min following *i.v.* administration of [$^{14}$C]creatinine. The blood concentration was not significantly different between the two groups of rats ($p>0.05$). However, the concentrations in the brain and myocardium of the decapitated rats were smaller than those of the frozen rats ($p<0.05$). A 2-fold difference was observed in the brain concentration. This might be brought about by the following two factors: (1) the concentration of creatinine in the brain and myocardium was considerably smaller than the blood concentration at this time after administration; (2) the blood contents in these tissues were different between the two groups of rats. One or both of the above two conditions might not hold in tissues other than brain and myocardium.

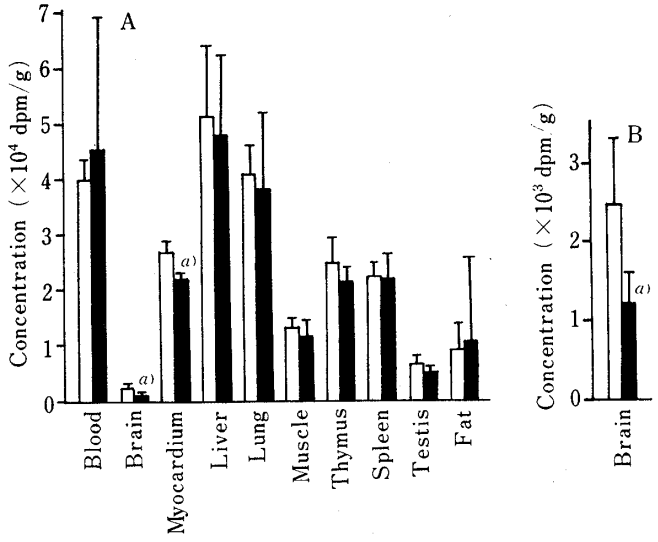


Fig. 1. Blood and Tissue Concentrations in Rats Sacrificed by Freezing or Decapitation at 5 min Following *i.v.* Administration of [$^{14}$C]-Creatinine

□, frozen rats; ■, decapitated rats.
Each result represents the mean ± S.D. for four to five rats. B is an enlarged version of the brain data in A.
*a*) Significantly different from the value for frozen rats at $p<0.05$.

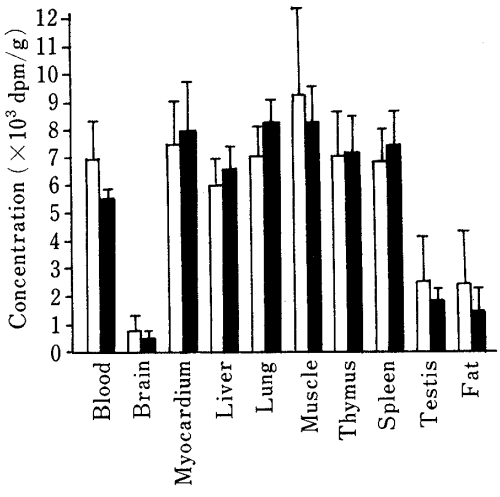


Fig. 2. Blood and Tissue Concentrations in Rats Sacrificed by Freezing or Decapitation at 60 min Following *i.v.* Administration of [$^{14}$C]-Creatinine

□, frozen rats; ■, decapitated rats.
Each result represents the mean ± S.D. for five rats.

Figure 2 shows the results at 60 min after administration. According to the plasma level–time data given in the previous paper,[1b] the approximate equilibrium for creatinine distribution was assumed to be attained between plasma and various tissues at this time. No significant difference was observed in any tissue, including the brain and myocardium ($p > 0.05$). The decrease of the difference in creatinine concentration between the blood and brain or myocardium was assumed to be responsible for this.

The above results indicate that the experimental method for collecting tissues does affect the brain and myocardium concentrations in rats administered [$^{14}$C]creatinine. The blood contents of tissues in the rats remain to be clarified.

**References**

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