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Effect of Convulsions Induced by Pentylenetetrazole or Electricity on the Dispositions of Creatinine and Urea in Rats[1)]

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The effect of convulsions on the dispositions of creatinine and urea, which are considered to pass through the water-filled pores of biological membranes easily, was examined by means of plasma analysis and whole-body autoradiography following intravenous administration of each compound to rats during tonic convulsions induced by pentylenetetrazole or electricity.

The data on the plasma levels showed that, in convulsed rats, the transfer of creatinine and urea from plasma to tissues was suppressed and the total body clearance of both chemicals was significantly decreased. The results of whole-body autoradiography, which showed decreased or almost stopped blood flow in the intestinal tract, skin, and kidney of convulsed rats, were in good agreement with the above observations.

Keywords——creatinine; urea; convulsed rat; pentylenetetrazole; electricity; plasma level; whole-body autoradiography; disposition; total body clearance

There are few reports dealing with the absorption, distribution, metabolism and excretion of drugs in animals in model disease states, especially in regard to drug distribution. In the preceding papers,[2-4)] the authors reported the distributions of creatinine and urea, which are considered to pass through the water-filled pores of biological membranes easily, in nephrectomized rats, hereditary muscular dystrophic mice and hyperthyroid mice.

Convulsions are well known as major symptoms of epilepsy, but are also induced by infections and tumors in the brain, abnormalities in metabolism, poisonings, *etc.* Although many papers on convulsions have appeared, most are concerned with aspects of biochemistry and pharmacology,[5-7)] and little work has been done on the effect of convulsions on the biopharmaceutic and pharmacokinetic properties of drugs. In this paper, plasma analysis and whole-body autoradiography for creatinine and urea following intravenous administration to rats during tonic convulsions induced by pentylenetetrazole (CNS stimulant) or electricity were carried out to clarify how the elimination and distribution of drugs are changed by convulsions.

Materials and Methods

Chemicals——[carbonyl-^{14}C]Creatinine hydrochloride (specific activity, 12.0 mCi/mmol) and ^{14}C-urea (specific activity, 9.6 mCi/mmol) were purchased from Amersham International, Amersham, England, and New England Nuclear, Boston, Mass., U.S.A., respectively. The radiochemical purity of each chemical was greater than 98%. All other chemicals were of analytical grade and were used without further purification.

Animals——Male Wistar rats, 6 to 7 weeks old, were purchased from Shizuoka Agricultural Cooperative for Experimental Animals, Hamamatsu, Japan, for use in all experiments. 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.[8)]

Induction of Tonic Convulsions by Pentylenetetrazole——A saline solution of pentylenetetrazole (Aldrich

Chemical Company, Milwaukee, Wis., U.S.A.) was administered intraperitoneally to rats at a dose of 80 mg/1 ml/kg. Control rats were given saline (1 ml/kg, sham injection) intraperitoneally. Most of the rats given pentylenetetrazole showed the symptoms of tonic convulsions within a few minutes after the administration, and died within 15 min.

Induction of Tonic Convulsions by Electricity——Rats were administered saline intraperitoneally (1 ml/kg, sham injection) and both ears were fitted with metal clips which were connected to a source of electricity (130 V). Rats were then charged for 2 s after wetting of the ears with saline. All rats showed the symptom of tonic convulsions immediately, but recovered after the electric stimulus ceased.

Plasma Levels of ^{14}C-Creatinine and ^{14}C-Urea Following Intravenous Administration——Rats were given 10 μCi/kg of ^{14}C-creatinine (942 μg/kg as creatinine) and ^{14}C-urea (626 μg/kg as urea) intravenously into the external jugular vein immediately after the induction of tonic convulsions by the above procedures. Control rats were also given these two chemicals following intraperitoneal administration of saline (1 ml/kg). Blood samples (250 μl) were withdrawn periodically into small heparinized and ice-cooled tubes. Plasma samples (100 μl) were obtained by centrifuging the tubes at 3000 rpm for 15 min and then were dissolved in 0.5 ml of Soluene-350 (Packard Instrument Co., Downers Grove, Ill., U.S.A.).

Whole-Body Autoradiography Following Intravenous Administration——Rats were given 100 μCi/kg of ^{14}C-creatinine (942 μg/kg as creatinine) and ^{14}C-urea (626 μg/kg as urea) intravenously into the external jugular vein immediately after the induction of tonic convulsions by the above procedures. Control rats were also given these two chemicals following intraperitoneal administration of saline (1 ml/kg). These rats were sacrificed at 30 s following the administration of each radiochemical by soaking them in dry ice–acetone (-78 °C) without any anesthesia. Sections (40 μm) were obtained with a microtome (Yamato 1111, Tokyo, Japan) at about -25 °C, and attached to SALOTAPE (Hisamitsu Pharmaceutical Co., Ltd., Tosu, Japan). After being dried in a freeze-dryer for a few days, the sections were placed in contact with X-ray films (No. 150, Fuji Photo Film Co., Ltd., Tokyo, Japan) for 20 d at 4 °C.

Radioactivity Measurement——The radioactivity was determined in a Mark II liquid scintillation spectrometer (Nuclear-Chicago Corporation, Des Plaines, Ill., U.S.A.). All samples were determined with 10 ml of toluene–Triton X-100 liquid scintillator (PPO 5 g, POPOP 300 mg, 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

Plasma Levels of ^{14}C-Creatinine and ^{14}C-Urea Following Intravenous Administration to Rats during Tonic Convulsions Induced by Pentylenetetrazole

The induction of convulsions by pentylenetetrazole is not due to a direct action on the organs or tissues,[9] and the mechanism is different from that in the case of electric stimulus.[10]

Creatinine is not metabolized in rats[11] (though the microflora in the gastrointestinal tract can metabolize creatinine[12]) and is quantitatively excreted into the urine following intravenous administration to rats.[12] Therefore, all of the radioactivity in plasma obtained following intravenous administration of ^{14}C-creatinine was regarded as originating from ^{14}C-

Table I. Plasma Levels of ^{14}C-Creatinine and ^{14}C-Urea Following Intravenous Administration to Rats during Tonic Convulsions Induced by Pentylenetetrazole and Levels in the Control

Chemical	Time (min)	Plasma level ($\times 10^4$ dpm/ml)	
		Convulsed	Control
Creatinine	2	17.1[a] ± 4.6[b] (3)[c]	5.8 ± 0.2 (3)
	5	10.3[a] ± 0.9 (4)	4.4 ± 0.2 (4)
	10	7.9 ± 0.3 (2)	3.2 ± 0.0 (3)
Urea	2	6.5[a] ± 0.6[b] (4)[c]	4.2 ± 0.4 (3)
	5	4.5[a] ± 0.3 (3)	3.1 ± 0.2 (3)

a) Significantly different from the control value ($p<0.01$).
b) Standard deviation.
c) The number in parenthesis indicates the number of rats.

creatinine. On the other hand, about 12% of ^{14}C-urea administered intravenously to rats is metabolized and excreted as $^{14}CO_2$ into the expired air.[13] However, in this paper, all of the radioactivity in plasma obtained following intravenous administration of ^{14}C-urea was provisionally regarded as originating from ^{14}C-urea.

The plasma level-time data for ^{14}C-creatinine following intravenous administration to rats during tonic convulsions induced by pentylenetetrazole and the control values are summarized in Table I. As rats died within 15 min after the induction of tonic convulsions, comparison of pharmacokinetic parameters was not feasible. However, the plasma levels at 2 and 5 min in convulsed rats were significantly ($p<0.01$) and considerably (2 to 3 times) higher than the corresponding levels in the control, suggesting that the transfer of creatinine from plasma to tissues was suppressed by convulsions. The change of blood flow rate in various tissues as a result of convulsions might be responsible for this result, as will be mentioned later.

A similar, but less pronounced, result was obtained with urea (Table I). Plasma levels at 2 and 5 min following intravenous administration of ^{14}C-urea to convulsed rats were about 1.5 times the corresponding levels in the control ($p<0.01$).

Plasma Levels of ^{14}C-Creatinine and ^{14}C-Urea Following Intravenous Administration to Rats during Tonic Convulsions Induced by Electricity

Figure 1 shows the plasma level–time data for ^{14}C-creatinine following intravenous administration to rats during tonic convulsions induced by electricity, together with those for the control. The rats did not die of the convulsions induced by electricity, in contrast to the case of those induced by pentylenetetrazole, and the pharmacokinetic parameters were estimated on the basis of a two-compartment open model (Table II). Significant ($p<0.01$) but relatively small differences were observed in all the parameters. The distribution volume, *i.e.*, $(V_d')_{extrap}$ and $(V_d')_\beta$, for convulsed rats was about 0.4—0.5 times the value for the control, in other words, the transfer of creatinine from plasma to tissues was suppressed in convulsed rats compared to the control. The total body clearance ($k_{10} \cdot V_1$) was also smaller in convulsed rats (9.3 ± 0.6 ml/min/kg) than in the control (13.0 ± 1.5 ml/min/kg). This result suggests that renal clearance of creatinine,which is generally accepted as an index of renal function in man, is

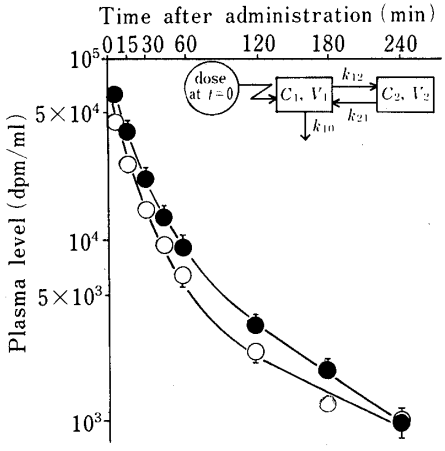

Fig. 1. Plasma Levels of ^{14}C-Creatinine Following Intravenous Administration to Rats during Tonic Convulsions Induced by Electricity (●), and Levels in the Control (○)

Each point represents the mean ± S.D. for three to four rats. The points without a vertical bar have S.D. values smaller than the circles. The plots are computer-fitted curves (weight $(i)=1/C_i^2$).

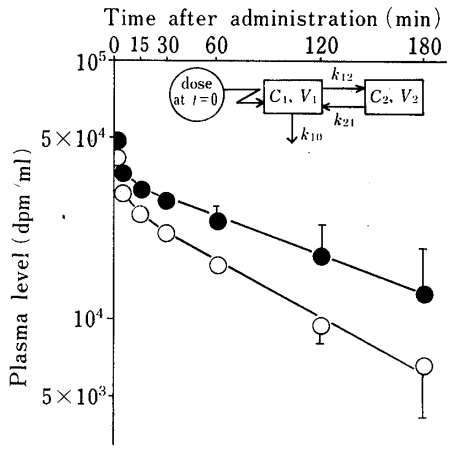

Fig. 2. Plasma Levels of ^{14}C-Urea Following Intravenous Administration to Rats during Tonic Convulsions Induced by Electricity (●), and Levels in the Control (○)

Each point represents the mean ± S.D. for three to four rats. The points without a vertical bar have S.D. values smaller than the circles. The plots are computer-fitted curves (weight $(i)=1/C_i^2$).

TABLE II. Pharmacokinetic Parameters for ^{14}C-Creatinine Following Intravenous Administration to Rats during Tonic Convulsions Induced by Electricity (Value for Parameter ± Standard Error[a])

Parameter	Convulsed rats ($n=10$)[b]	Control ($n=10$)
A (dpm/ml)	7.18×10^{4}[c] $\pm3.85\times10^{3}$	4.82×10^{4} $\pm4.04\times10^{3}$
B (dpm/ml)	1.22×10^{4}[c] $\pm1.73\times10^{3}$	4.95×10^{3} $\pm1.62\times10^{3}$
α (min^{-1})	5.84×10^{-2}[c] $\pm4.59\times10^{-3}$	$4.84\times10^{-2}\pm5.47\times10^{-3}$
β (min^{-1})	1.05×10^{-2}[c] $\pm7.53\times10^{-4}$	$7.01\times10^{-3}\pm1.70\times10^{-3}$
k_{10} (min^{-1})	3.51×10^{-2}[c] $\pm1.52\times10^{-3}$	$3.12\times10^{-2}\pm2.44\times10^{-3}$
k_{12} (min^{-1})	1.63×10^{-2}[c] $\pm2.27\times10^{-3}$	$1.33\times10^{-2}\pm2.30\times10^{-3}$
k_{21} (min^{-1})	1.75×10^{-2}[c] $\pm2.02\times10^{-3}$	$1.09\times10^{-2}\pm3.07\times10^{-3}$
V_1' (ml/kg)	2.64×10^{2}[c] $\pm1.28\times10^{1}$	4.18×10^{2} $\pm3.33\times10^{1}$
V_2' (ml/kg)	2.47×10^{2}[c] $\pm4.62\times10^{1}$	5.11×10^{2} $\pm1.74\times10^{2}$
$(V_d')_{extrap}$ (ml/kg)[d]	1.82×10^{3}[c] $\pm2.58\times10^{2}$	4.48×10^{3} $\pm1.47\times10^{3}$
$(V_d')_\beta$ (ml/kg)[e]	8.83×10^{2}[c] $\pm8.54\times10^{1}$	1.86×10^{3} $\pm4.97\times10^{2}$
AUC (dpm·min/kg)	2.39×10^{6}[c] $\pm2.19\times10^{5}$	1.70×10^{6} $\pm3.19\times10^{5}$
$t_{1/2\beta}$ (min)	6.60×10^{1}[c] $\pm4.73\times10^{0}$	9.89×10^{1} $\pm2.40\times10^{1}$
$k_{10}\cdot V_1'$ (ml/min/kg)	9.27×10^{0}[c] $\pm6.02\times10^{-1}$	1.30×10^{1} $\pm1.46\times10^{0}$

a) W. E. Deming "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946.
b) The number of input data.
c) Significantly different from the control value at $p<0.01$.
d) $(V_d')_{extrap}=(dose)/B$.
e) $(V_d')_\beta=V_1'\cdot k_{10}/\beta$.

TABLE III. Pharmacokinetic Parameters for ^{14}C-Urea Following Intravenous Administration to Rat during Tonic Convulsions Induced by Electricity (Value for Parameter ± Standard Error[a])

Parameter	Convulsed rats ($n=8$)[b]	Control ($n=8$)
A (dpm/ml)	3.66×10^{4} $\pm4.05\times10^{3}$	2.93×10^{4} $\pm9.77\times10^{3}$
B (dpm/ml)	3.38×10^{4}[c] $\pm3.09\times10^{2}$	2.73×10^{4} $\pm1.23\times10^{3}$
α (min^{-1})	4.47×10^{-1}[d] $\pm4.89\times10^{-2}$	$3.40\times10^{-1}\pm1.31\times10^{-1}$
β (min^{-1})	5.47×10^{-3}[c] $\pm9.36\times10^{-5}$	$8.21\times10^{-3}\pm4.51\times10^{-4}$
k_{10} (min^{-1})	1.13×10^{-2}[c] $\pm6.87\times10^{-4}$	$1.66\times10^{-2}\pm3.01\times10^{-3}$
k_{12} (min^{-1})	2.24×10^{-1} $\pm3.57\times10^{-2}$	$1.64\times10^{-1}\pm8.81\times10^{-2}$
k_{21} (min^{-1})	2.18×10^{-1}[c] $\pm1.41\times10^{-2}$	$1.68\times10^{-1}\pm4.53\times10^{-2}$
V_1' (ml/kg)	3.15×10^{2}[c] $\pm1.86\times10^{1}$	3.92×10^{2} $\pm7.04\times10^{1}$
V_2' (ml/kg)	3.24×10^{2}[c] $\pm5.91\times10^{1}$	3.82×10^{2} $\pm2.40\times10^{2}$
$(V_d')_{extrap}$ (ml/kg)[e]	6.57×10^{2}[c] $\pm6.01\times10^{0}$	8.13×10^{2} $\pm3.66\times10^{1}$
$(V_d')_\beta$ (ml/kg)[f]	6.52×10^{2} $\pm5.63\times10^{1}$	7.92×10^{2} $\pm2.07\times10^{2}$
AUC (dpm·min/ml)	6.26×10^{6}[c] $\pm1.21\times10^{5}$	3.42×10^{6} $\pm2.40\times10^{5}$
$t_{1/2\beta}$ (min)	1.27×10^{2}[c] $\pm2.17\times10^{0}$	8.44×10^{1} $\pm4.64\times10^{0}$
$k_{10}\cdot V_1'$ (ml/min/kg)	3.56×10^{0}[c] $\pm3.02\times10^{-1}$	6.51×10^{0} $\pm1.66\times10^{0}$

a) W. E. Deming "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946.
b) The number of input data.
c) Significantly different from the control value at $p<0.01$.
d) Significantly different from the control value at $p<0.05$.
e) $(V_d')_{extrap}=(dose)/B$.
f) $(V_d')_\beta=V_1'\cdot k_{10}/\beta$.

decreased by convulsions, and it apparently did not recover to the normal level, at least during the blood sampling period used in this experiment.

Figure 2 shows the plasma level–time data for ^{14}C-urea, and the estimated pharmacokinetic parameters on the basis of a two-compartment open model are summarized in Table III. In line with the case of creatinine, significant ($p<0.05$ or $p<0.01$) but relatively small differences were observed in almost all parameters. The total body clearances in convulsed rats and the control were 3.6 ± 0.3 and 6.5 ± 1.7 ml/min/kg, respectively. This might reflect decreased renal clearance of urea as a result of convulsions, as in the case of creatinine.

The data for the control rats in this experiment were compatible with our previous results on the distributions of creatinine[12] and urea[13] in normal rats.

Whole-Body Autoradiography Following Intravenous Administration

Figure 3 shows the whole-body autoradiograms at 30 s following intravenous administration of ^{14}C-creatinine to rats during tonic convulsions induced by pentylenetetrazole (Fig. 3-A) or electricity (Fig. 3-B), together with the autoradiogram for the control (Fig. 3-C). Uneven distributions of radioactivity were apparent within the muscle, skin and intestinal wall (*i.e.*, different regions within a tissue contained very different levels of radioactivity) as shown in Figs. 3-A and 3-B, especially in Fig. 3-A, in contrast to the distribution pattern in

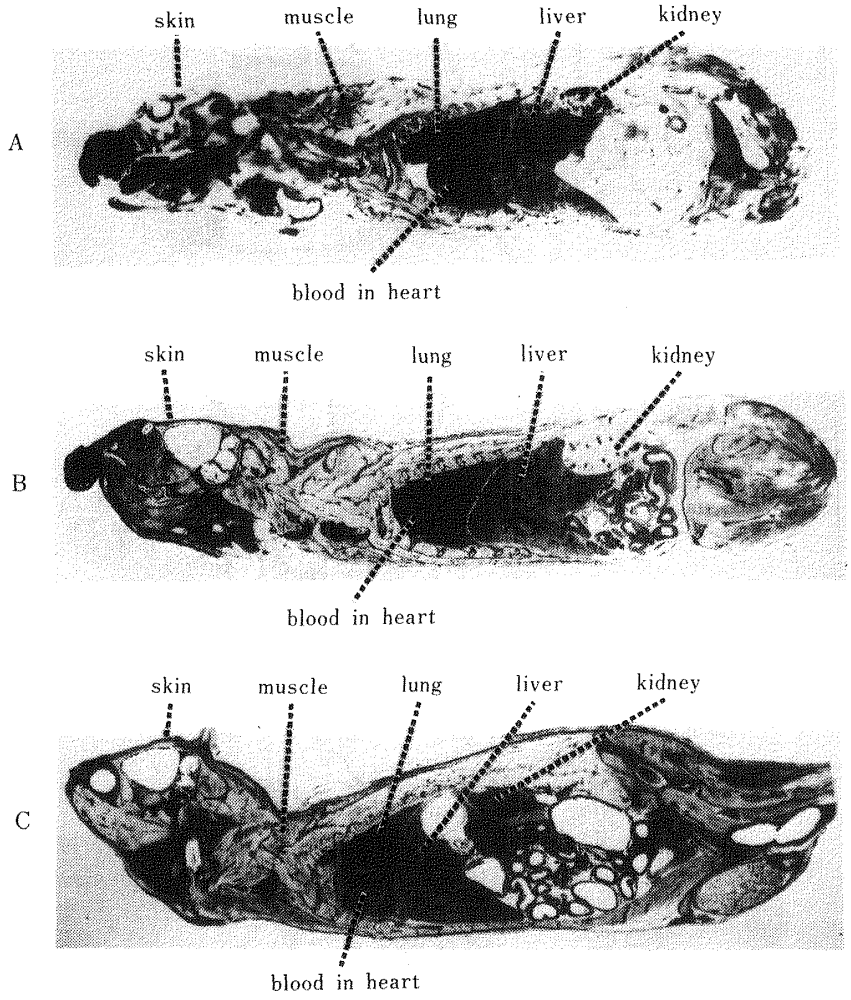

Fig. 3. Whole-Body Autoradiograms Showing the Distribution of Radioactivity (Dark Areas) at 30 s Following Intravenous Administration of ^{14}C-Creatinine to Rats during Tonic Convulsions Induced by Pentylenetetrazole (A) or Electricity (B). Control (C).

Fig. 3-C, which is nearly homogeneous within each tissue. Such an uneven distribution pattern within each tissue of convulsed rats can be explained by the assumptions that different regions within each tissue are perfused by blood in different blood vessel systems and that the extents of change of the blood flow rate caused by convulsions differ in different blood vessel systems.

Most parts of the skin (Figs. 3-A and 3-B) and intestinal wall (Fig. 3-A) of convulsed rats did not contain any radioactivity, in other words, the blood flow might have completely stopped there.

The transfer of radioactivity to the kidney was also changed considerably by convulsions. In convulsed rats, the radioactivity contained in the kidney was small (especially in renal medulla: Fig. 3-A) or almost negligible (Fig. 3-B) in contrast to the homogeneous and high-level distribution in the control. This result suggests that the renal blood flow rate is also considerably decreased by convulsions. This autoradiographic result for the kidney corresponds well to the decreased creatinine total body clearance in convulsed rats, as mentioned above.

Figure 4 shows the results for urea at 30 s following intravenous administration to rats during tonic convulsions induced by pentylenetetrazole (Fig. 4-A) or electricity (Fig. 4-B),

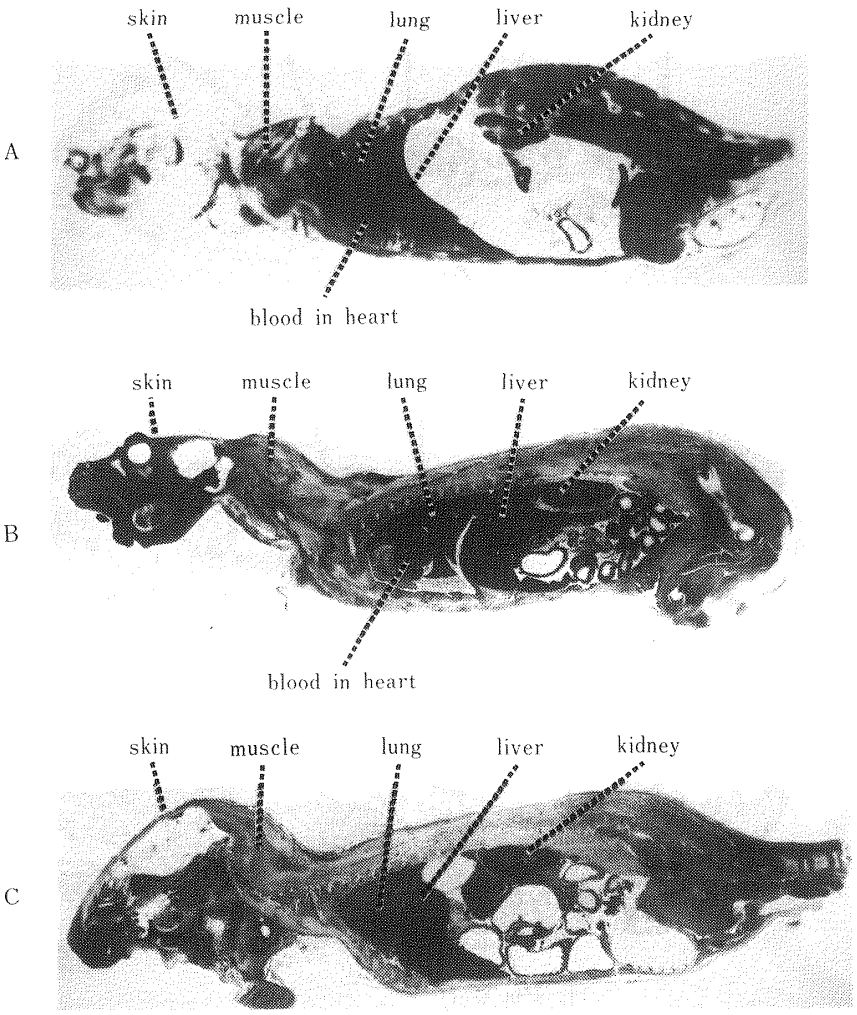

Fig. 4. Whole-Body Autoradiograms Showing the Distribution of Radioactivity (Dark Areas) at 30 s Following Intravenous Administration of ^{14}C-Urea to Rats during Tonic Convulsions Induced by Pentylenetetrazole (A) or Electricity (B). Control (C).

together with the autoradiogram for the control (Fig. 4-C). The change of urea distribution pattern caused by convulsions was similar to that in the case of creatinine, but urea was distributed at considerably high levels in the muscle at the back (Fig. 4-A) and in the kidney (Fig. 4-B) in contrast to the case of creatinine (Figs. 3-A and 3-B). This seems to correspond well to the results mentioned above that the plasma levels at 2 and 5 min (pentylenetetrazole stimulus) and the distribution volume (electric stimulus) of urea were not very different from those in the control, as in the case of creatinine. The above observation might be related to the fact that urea is more diffusible than creatinine in the body.[12)]

The changes of creatinine and urea distributions to the kidney, intestinal wall, muscle, *etc.* by convulsions were different in the cases of pentylenetetrazole and electric stimulus, and were larger in the case of pentylenetetrazole stimulus (Figs. 3-A and 4-A).

In this work, in order to clarify how the elimination and distribution of drugs are changed by convulsions, plasma analysis and whole-body autoradiography for creatinine and urea given intravenously to rats during tonic convulsions induced by pentylenetetrazole or electricity were carried out. However, physiological changes such as peripheral blood flow rate, blood pressure, *etc.* were not determined because of the technical difficulties. If these difficulties can be overcome, it would be interesting to compare the results obtained in this study with measures of peripheral blood flow rate or blood pressure.

References and Notes

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