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Absorption and Distribution of Creatinine and Urea in Hereditary Muscular Dystrophic Mice[1)]

JUN WATANABE,* JUNJI HIRATE, KIKUO IWAMOTO, and SHOJI OZEKI

Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

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The absorption and distribution of creatinine and urea, which are considered to pass through the water-filled pores of biological membranes easily, were investigated in hereditary muscular dystrophic mice as model animals afflicted with a disease which changes the structure and function of the cell membrane.

The $V_d'_{extrap}$ for creatinine was remarkably larger in dystrophic mice than that of the control. Whole-body autoradiograms showed that creatinine was distributed rather more uniformly throughout the body in dystrophic mice than in the control at 2 min. A similar, but less pronounced, tendency was also observed in the case of urea.

For both chemicals, the urinary excretion of radioactivity in 24 h following oral administration was significantly larger in dystrophic mice than in the control ($p<0.02$).

The above results indicated that the membrane permeability to these two chemicals was enhanced by the disease.

Keywords——creatinine; urea; muscular dystrophy; distribution; absorption; autoradiography; enhanced membrane permeability

There are few reports dealing with the absorption, distribution, metabolism and excretion of drugs in animals with model disease states, especially as regards drug distribution.

The disposition of creatinine and urea in nephrectomized rats (a model of renal failure) was described in our preceding publication.[1)] In this report, hereditary muscular dystrophic mice, which are known to show close pathological similarity to human muscular dystrophy, were used as model animals of a disease state in which the structure and function of the cell membrane may be altered.

Human muscular dystrophy is a disease accompanied by abnormal enzyme transfer from the cell into the blood stream, and this transfer is especially marked for enzymes such as creatine phosphokinase and aldolase.[2,3)] Similar pathological changes have been observed in dystrophic mice.[4)] Muscular dystrophy is likely to cause structural change in the cell membrane and consequently may alter the permeability to various substances.[5-7)] However, no change in the distribution of exogeneous substances during this disease has yet been demonstrated.

In this paper, the distribution of creatinine and urea, which presumably pass through the water-filled pores of biological membranes easily, was investigated by blood analysis and whole-body autoradiography following intravenous administration. Intestinal permeability to these two compounds was also elucidated by measurements of urinary, fecal and expiratory excretion following oral administration.

Materials and Methods

Animals——Male C57 BL/6j-dy strain dystrophic mice (dy/dy) and the control (+/?) were obtained from the Central Laboratory of Experimental Animals, Kawasaki, Japan and mice of 5 to 14 weeks old were used.

Chemicals——[carbonyl-^{14}C]Creatinine hydrochloride (specific activity, 12.0 mCi/mmol) and ^{14}C-urea (specific activity, 8.6 mCi/mmol) were purchased from The Radiochemical Centre Amersham, England, and

New England Nuclear, Boston, Mass., U.S.A., respectively. The radiochemical purity of each chemical was more than 99%. ^{3}H-Water (specific activity, 18.0 mCi/mol) was also purchased from New England Nuclear. All other chemicals were of analytical grade and were used without further purification.

Experimental Procedures——(1) Whole Blood Levels of ^{14}C-Creatinine, ^{14}C-Urea and ^{3}H-Water following Intravenous Administration: Mice were given 100 μCi/kg of ^{14}C-creatinine (942 μg/kg as creatinine) and ^{14}C-urea (699 μg/kg as urea) or 1 mCi/kg of ^{3}H-water (6.7 or 7.4 ml/kg as water) into the tail vein. Blood samples (5 μl) were taken periodically with MICROCAPS (Drummond Scientific Co., Broomall, Pa., U.S.A.) by wounding the tail vein with a razor.

(2) Whole-body Autoradiography following Intravenous Administration of ^{14}C-Creatinine and ^{14}C-Urea: Mice were given 100 μCi/kg of ^{14}C-creatinine (942 μg/kg as creatinine) and ^{14}C-urea (699 μg/kg as urea) into the tail vein, and sacrificed at 30 s and 2 min following the administration by soaking them in dry ice-acetone (−78°C) without anesthesia. Sections (40 μm) were obtained with a microtome (Yamato 1111, Tokyo, Japan) at about −25°C, and attached to SALOTAPE (Hisamitsu Seiyaku Co. Ltd., Thosu, Japan). After being dried in a freeze-dryer for a few days, the sections were contacted with X-ray films (No. 150, Fuji Photo Film Co. Ltd., Tokyo, Japan) for 20 days at 4°C.

(3) Urinary, Fecal and Expiratory Excretion of Radioactivity following Oral Administration of ^{14}C-Creatinine and ^{14}C-Urea: Mice were fasted for over 15 h before experiments, and given 200 μCi/kg of ^{14}C-creatinine (1.9 mg/kg as creatinine) and ^{14}C-urea (1.4 mg/kg as urea) by gastric intubation. The mice were then housed individually in metabolic cages (KN-450, Natsume, Tokyo, Japan), which were equipped to capture $^{14}CO_2$ in a mixture of ethanolamine and methanol (1: 2, v/v).

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 standardization method and cpm was converted to dpm.

Results and Discussion

Whole Blood Levels of ^{14}C-Creatinine, ^{14}C-Urea and ^{3}H-Water following Intravenous Administration

Whole blood elimination curves of ^{14}C-creatinine following intravenous administration to dystrophic mice and the control, which were analyzed on the basis of a two-compartment open model, are shown in Fig. 1, and the estimated pharmacokinetic parameters are summarized in Table I. When the parameters for dystrophic mice were compared with those of the control, A, B, α, β, k_{10} and k_{21} were significantly smaller and V_2 was significantly larger in dystrophic mice ($p<0.01$). Since a remarkable difference was observed in B, $V_{d'extrap}$ for dystrophic mice (2.39×10^{4} ml/kg) was about six times that of the control (3.72×10^{3} ml/kg).

A similar, but less pronounced, tendency was also found in the case of urea, for which the whole blood elimination data were analyzed according to a one-compartment open model. The results for urea are shown in Fig. 2 and Table II. The V_{d}' and k_{el} for dystrophic mice were significantly larger than the control values ($p<0.01$), but the differences were small.

It was evident that the distribution volumes of these two chemicals in mice were increased by affliction with muscular dystrophy. This might occur as a result of

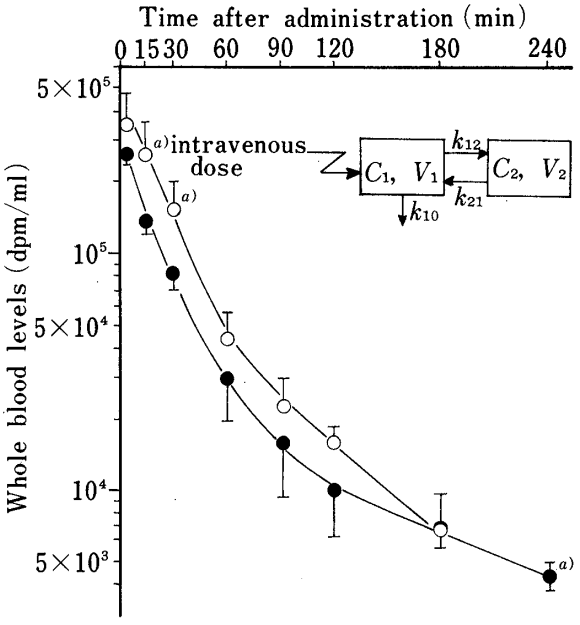

Fig. 1. Whole Blood Levels of ^{14}C-Creatinine following Intravenous Administration to Dystrophic Mice (●) and the Control (○)

Each point represents the mean ± S.D. for three to six animals (*a*: Mean with range for two animals).
Dose: 100 μCi/kg (942 μg/kg as creatinine).

TABLE I. Pharmacokinetic Parameters for ^{14}C-Creatinine following Intravenous Administration to Dystrophic Mice and the Control (Value for Parameter ± Standard Error[a])

Parameter	Dystrophic mice ($n=46$)	Control ($n=27$)
A, dpm/ml	2.74×10^{5} [d] $\pm2.25\times10^{4}$	3.42×10^{5} $\pm4.76\times10^{4}$
B, dpm/ml	9.29×10^{3} [d] $\pm4.09\times10^{3}$	5.96×10^{4} $\pm3.28\times10^{4}$
α, min^{-1}	4.47×10^{-2} [d] $\pm4.12\times10^{-3}$	$5.33\times10^{-2}\pm1.44\times10^{-2}$
β, min^{-1}	3.24×10^{-3} [d] $\pm2.37\times10^{-3}$	$1.21\times10^{-2}\pm3.41\times10^{-3}$
k_{10}, min^{-1}	3.15×10^{-2} [d] $\pm5.34\times10^{-3}$	$3.54\times10^{-2}\pm4.82\times10^{-3}$
k_{12}, min^{-1}	1.19×10^{-2} $\pm3.14\times10^{-3}$	$1.17\times10^{-2}\pm5.98\times10^{-3}$
k_{21}, min^{-1}	4.59×10^{-3} [d] $\pm2.94\times10^{-3}$	$1.82\times10^{-2}\pm7.71\times10^{-3}$
V_1, ml/kg	7.84×10^{2} $\pm6.60\times10^{1}$	5.53×10^{2} $\pm7.54\times10^{1}$
V_2, ml/kg	2.02×10^{3} [d] $\pm1.41\times10^{3}$	3.56×10^{2} $\pm2.41\times10^{2}$
AUC, dpm·min/ml	9.00×10^{6} [d] $\pm2.56\times10^{6}$	1.14×10^{7} $\pm3.62\times10^{6}$
$(V_d')_{extrap}$, ml/kg[b]	2.39×10^{4} [d] $\pm1.05\times10^{4}$	3.72×10^{3} $\pm2.05\times10^{3}$
$(V_d')_{\beta}$, ml/kg[c]	7.61×10^{3} [d] $\pm5.76\times10^{3}$	1.62×10^{3} $\pm5.52\times10^{2}$
$t_{1/2\beta}$, h	3.56[d] ±2.62	0.95 ±0.27

a) According to the method in the textbook "Statistical Adjustment of Data," ed. by W.E. Deming, John Wiley and Sons, Inc., New York, 1946.
b) $(V_d')_{extrap}=(\text{Dose})/B$.
c) $(V_d')\beta=V_1\cdot k_{10}/\beta$.
d) Significantly different from control at $p<0.01$.

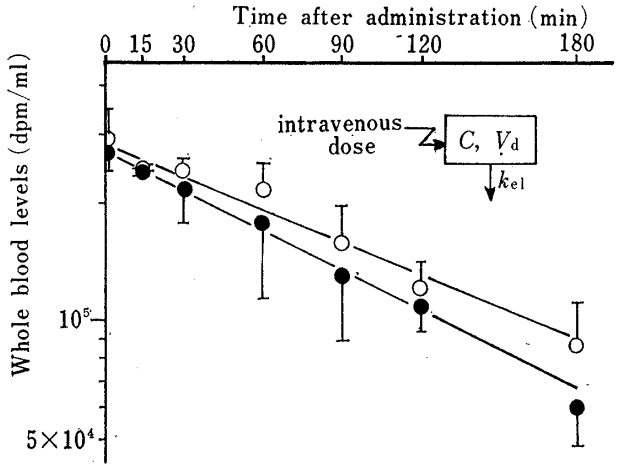

Fig. 2. Whole Blood Levels of ^{14}C-Urea following Intravenous Administration to Dystrophic Mice (●) and the Control (○)

Each point represents the mean ± S. D. for three to six animals. Dose: 100 μCi/kg (699 μg/kg as urea).

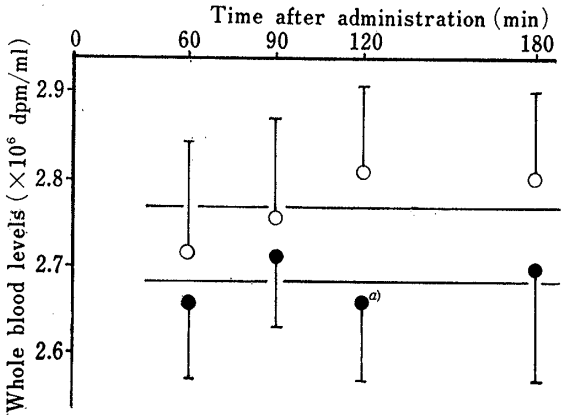

Fig. 3. Whole Blood Levels of ^{3}H-Water following Intravenous Administration to Dystrophic Mice (●) and the Control (○)

Each point represents the mean ± S.D. for three to four animals. (a: Mean with range for two animals). Dose: 1 mCi/kg.

TABLE II. Pharmacokinetic Parameters for ^{14}C-Urea following Intravenous Administration to Dystrophic Mice and the Control (Value for Parameter ± Standard Error[a])

Parameter	Dystrophic mice ($n=35$)	Control ($n=40$)
k_{el}, min^{-1}	7.57×10^{-3} [b] $\pm8.26\times10^{-4}$	$6.24\times10^{-3}\pm6.25\times10^{-4}$
V_d', ml/kg	8.33×10^{2} [b] $\pm2.79\times10^{1}$	7.87×10^{2} $\pm2.22\times10^{1}$
$t_{1/2}$, h	1.53[b] ±0.17	1.85 ±0.19

a) According to the method in the textbook "Statistical Adjustment of Data," ed. by W.E. Deming, John Wiley and Sons, Inc., New York, 1946.
b) Significantly different from control at $p<0.01$.

enhanced membrane permeability not only to such macro-molecules as creatine phosphokinase and aldolase but also to such small molecules as creatinine and urea. As has previously been shown in an analysis of the distribution pattern of these two chemicals by whole-body autoradiography,[12] it seems probable that urea (M.W.: 60.1) is more diffusible than creatinine (M.W.: 113.1). This might be a reason for the smaller difference in distribution volume between dystrophic mice and the control in the case of urea when compared with creatinine.

It has been documented that creatinine distributes in total body water,[8-10] and that the existing form of water in dystrophic mice is slightly different from the control.[11] The authors, therefore, investigated the difference of whole blood levels of ^{3}H-water between the two groups of mice following intravenous administration in order to examine the difference in the distribution profiles of body water. The results are shown in Fig. 3. The steady-state concentration was not significantly different between dystrophic mice and the control ($p>0.05$), but the mean value for dystrophic mice was smaller than that for the control.

Whole-body Autoradiography following Intravenous Administration of ^{14}C-Creatinine and ^{14}C-Urea

Whole-body autoradiograms at 30 s and 2 min following intravenous administration of ^{14}C-creatinine are shown in Fig. 4. In dystrophic mice, creatinine was distributed uniformly throughout the body(except for the central nervous system and gut contents). In the control, however, considerable difference in creatinine concentration between tissues such as muscle and blood was observed. This result was consistent with the prediction from the data on whole blood concentration, which indicated that the peripheral tissues/blood ratio in dystrophic mice was closer to unity than that in the control at the early stage following intravenous administration.

The autoradiograms for urea at 30 s and 2 min are shown in Fig. 5. As was predicted from the data on whole blood concentration, hardly any difference between dystrophic mice and the control could be seen in the autoradiograms.

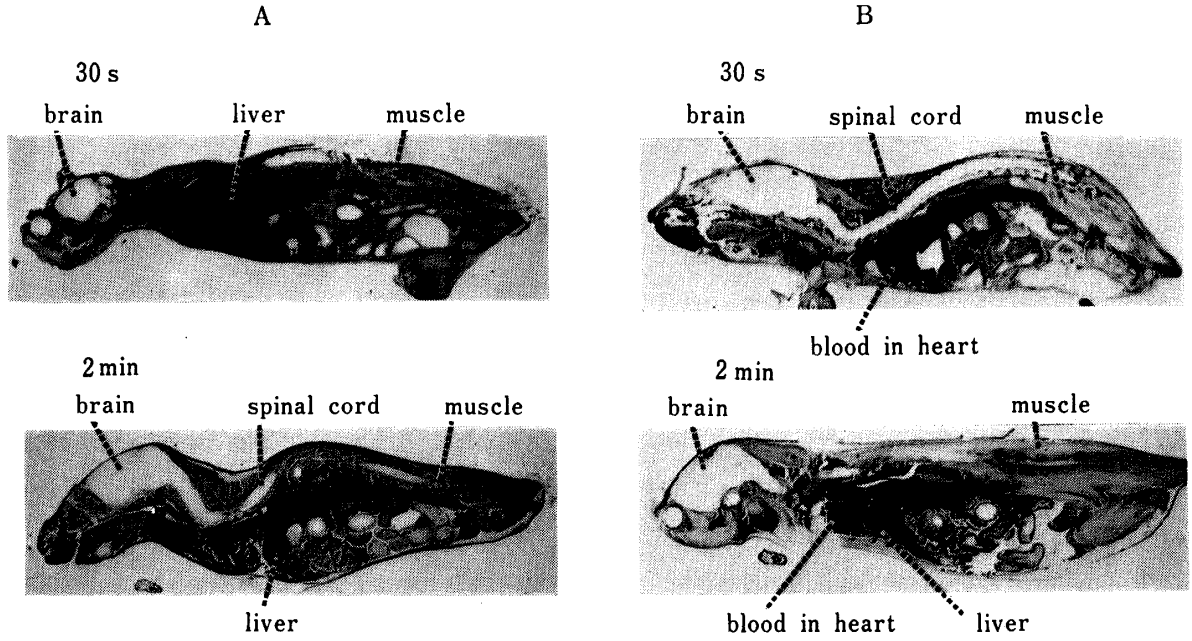

Fig. 4. Autoradiograms (30 s, 2 min) Showing the Distribution of Radioactivity (Dark Areas) following Intravenous Administration of ^{14}C-Creatinine to Dystrophic Mice (A) and the Control (B)

Dose: 100 μCi/kg (942 μg/kg as creatinine).

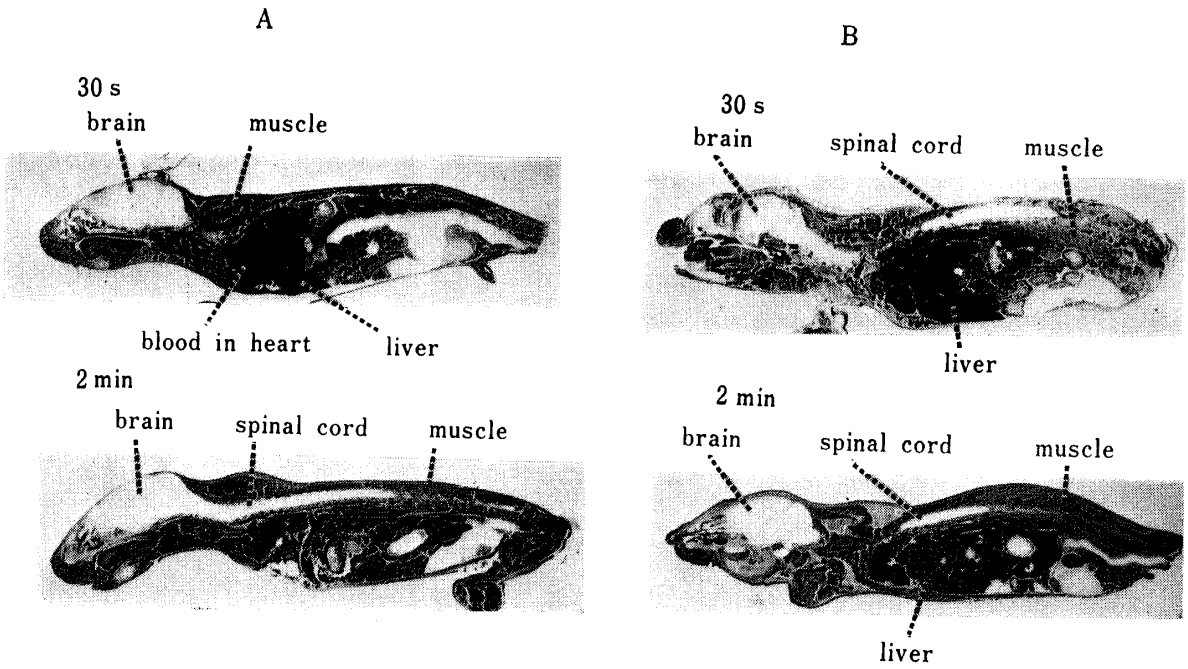

Fig. 5. Autoradiograms (30 s, 2 min) Showing the Distribution of Radioactivity (Dark Areas) following Intravenous Administration of ^{14}C-Urea to Dystrophic Mice (A) and the Control (B)

Dose: 100 μCi/kg (699 μg/kg as urea).

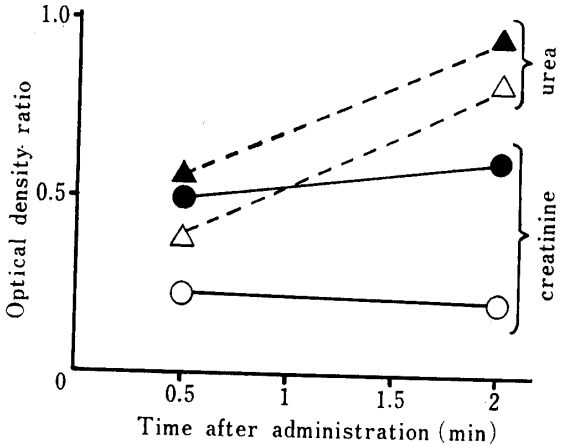

Fig. 6. Optical Density Ratio of Muscle to Heart Blood in Autoradiograms of Dystrophic Mice (●, ▲) and the Control (○, △) following Intravenous Administration of ^{14}C-Creatinine (●, ○) or ^{14}C-Urea (▲, △)

The optical density ratio of muscle to heart blood in these autoradiograms is shown in Fig. 6. For both chemicals, especially creatinine, the ratio in dystrophic mice was larger and closer to unity than that in the control at 30 s and 2 min. These results seem to correspond well to the above consideration based on visual observation of the autoradiograms. Moreover, it is clear that the ratio for urea approached unity more rapidly than that for creatinine. In other words, urea was more rapidly diffusible than creatinine.

Urinary, Fecal and Expiratory Excretion following Oral Administration of ^{14}C-Creatinine and ^{14}C-Urea

The results with creatinine and urea are shown in Table III and Table IV, respectively. For both chemicals, urinary excretion was the major excretion route of radioactivity and fecal excretion was negligible. Expiratory excretion of $^{14}CO_2$ was small following intravenous administration of [carbonyl-^{14}C]creatinine to rats,[12] and mice (ddY strain).[13] In the case of oral administration of creatinine, however, considerable expiratory excretion was observed, probably due to the absorption of creatinine metabolites which had been produced from unabsorbed creatinine by intestinal microflora.[1] Expiratory excretion of $^{14}CO_2$ following intravenous administration of ^{14}C-urea was about 11—12% in both rats and mice, and in the case of oral administration, it was increased by two to three times owing to the action of intestinal urease produced by intestinal microflora.[13,14] Accordingly, the longer these two

Table III. Urinary, Fecal and Expiratory Excretion of Radioactivity following Oral Administration of ^{14}C-Creatinine to Dystrophic Mice and the Control

	Recovery in 24 h (% of dose ± S.D.[a])			
	Urine	Feces	Expired air	Total
Dystrophic mice ($n=4$)	72.5[b] ±5.6	1.9±1.4	17.1±5.4	91.4±7.3
Control ($n=4$)	55.9 ±8.2	5.5±3.0	24.8±4.7	86.1±5.2

a) Standard deviation.
b) Significantly different from control at $p<0.02$.

Table IV. Urinary, Fecal and Expiratory Excretion of Radioactivity following Oral Administration of ^{14}C-Urea to Dystrophic Mice and the Control

	Recovery in 24 h (% of dose ± S.D.[a])			
	Urine	Feces	Expired air	Total
Dystrophic mice ($n=3$)	84.0[b] ±2.4	0.11[c] ±0.01	11.0±2.0	95.1±4.0
Control ($n=3$)	67.5 ±6.6	0.26 ±0.07	21.1±7.0	88.9±3.7

a) Standard deviation.
b) Significantly different from control at $p<0.02$.
c) Significantly different from control at $p<0.05$.

chemicals exist in the intestinal tract, the higher the expiratory excretion would be. Therefore, the gastrointestinal absorption of these two chemicals was temporarily evaluated not as the sum of urinary and expiratory excretion but as urinary excretion alone in this paper. As shown in Table III and Table IV, urinary excretion of both chemicals in 24 hours was significantly larger in dystrophic mice than the control ($p<0.02$). This result indicated that the membrane permeability of the intestine to creatinine and urea in mice had been enhanced by muscular dystrophy. This observation is consistent with that for whole blood concentration (Fig. 1, Fig. 2, Table I, Table II).

Cumulative expiratory excretion-time data for creatinine and urea are shown in Fig. 7. The difference between dystrophic mice and the control was not statistically significant ($p>0.05$), even though the mean value for dystrophic mice was smaller than that for the control with both chemicals. This result also indirectly suggests enhancement of the membrane permeability of the intestine to creatinine and urea in dystrophic mice, since the longer these two chemicals are present in the intestinsl tract, the higher the expiratory excretion would be. Interestingly, expiratory excretion in the case of creatinine occurred from one hour post-administration, and such a delay of expiratory excretion had also been observed in

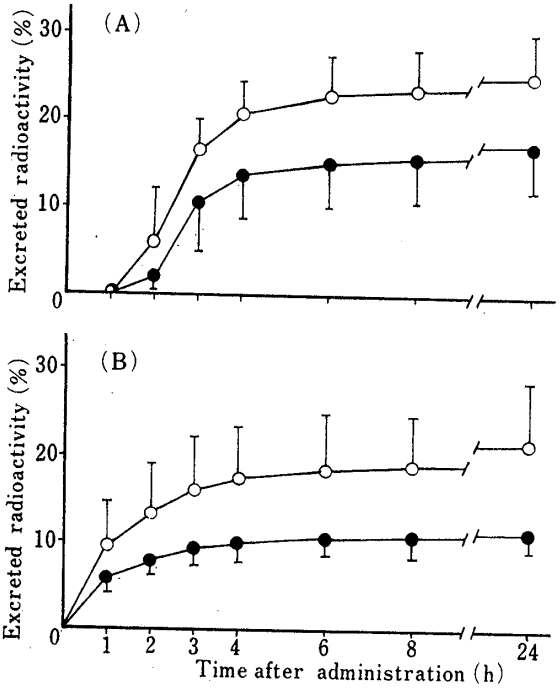

Fig. 7. Cumulative Expiratory Excretion of Radioactivity following Oral Administration of ^{14}C-Creatinine (A) and ^{14}C-Urea (B) to Dystrophic Mice (●) and the Control (○)

Dose: 200 μCi/kg (1.9 mg/kg as creatinine and 1.4 mg/kg as urea).

our previous study with rats.[12] Metabolic capacity for creatinine might exist in the distal portion of the intestine.

From these results on whole blood levels, whole-body autoradiography and urinary, fecal and expiratory excretion following intravenous and oral administration, it can be concluded that the membrane permeability to creatinine and urea is enhanced by muscular dystrophy.

References and Notes

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