After incubation the slices were quickly removed from the beakers, blotted and weighed. Both the tissue and a 2 ml aliquot of medium were assayed for PAH[11]. Results were expressed as slice/medium (S/M) ratio, where S equals mg/g of tissue and M equals mg/ml of medium. Data were analyzed statistically using Student's t-test, group or paired comparison. All data are expressed as the mean $\pm$ standard error. The 0.05 level of probability was used as the criterion of significance.

Results and discussion. Accumulation of PAH by renal cortical slices from the three species studied was dependent on the hydrogen ion concentration of the medium. PAH S/M ratio in rat tissue appeared to increase from a low at pH 7.5 to a sharp peak at pH 8.0 (Figure). Rabbit tissue also developed maximal PAH S/M ratios around pH 8.0 but the peak was not as sharp as that with rat tissue. In contrast, the PAH S/M ratio from dog tissue had a slight peak at pH 7.4 and then remained rather flat over the rest of the pH range studied.

Phosphate buffer was used over the entire pH range with tissue from the dog. When similar experiments were attempted with rat and rabbit tissues, the pH was not well maintained but returned toward 7.4. COPENHAVER and DAVIS[9] also noted that phosphate buffer was ineffective at higher pH when using rabbit kidney slices and therefore used a phosphate-propanediol combination buffer in their study. Propanediol buffer was used exclusively for rat and rabbit tissue in this study, nevertheless the buffering capacity of the slices still tended to shift the medium.

Ross et al.[10] observed that the amount of PAH accumulated during short (2–10 min) incubations was linear with time and suggested that this early accumulation is a reflection of the maximal rate at which PAH enters the slice. To determine the effect of medium pH on initial rate of uptake, PAH accumulation by rat renal cortical slices was determined in phosphate buffer initially adjusted to pH 7.5, 8.0 and 8.5. In 5 experiments no significant effect of medium pH was demonstrable until 30 min. After 10 min uptake ranged from 2.2–2.7 µg/100 mg tissue. At 15 min tissue at pH 7.4 had accumulated 4 µg/100 mg compared to 5 µg/100 mg in tissue at high pH. After 30 min tissue incubated initially at pH 8.5 had accumulated 8.8 $\pm$ 0.8 (S.E.) µg/100 mg which was significantly more than that at pH 8.0 7.1 $\pm$ 0.5) and pH 7.4 (6.6 $\pm$ 0.3). If uptake of PAH in the first few min of incubation truly reflects the rate of PAH transport these data demonstrate that the difference in pH alters some other aspect of the accumulation process, possibly an intracellular 'trapping' mechanism or diffusion from the tissue back into the medium. After incubating 30 min the tissue initially buffered to pH 8.5 had accumulated significantly more PAH than that at 7.4. Although the initial pH had declined to 7.8 during incubation, the slices had been functioning at a pH higher than 7.4 for 30 min. This, then, demonstrates that the enhanced PAH S/M seen at pH above 7.4 with propanediol (Figure) was a function of pH and not the buffer used[12].

Zusammenfassung. Nierenschnitte von verschiedenen Arten akkumulieren PAH mit zunehmendem pH-Wert des Mediums bei einem deutlichen Maximum zwischen pH 7.4 und pH 8.0.

H. M. BOWMAN[13], G. H. HIRSCH[14] and J. B. HOOK

Michigan State University, Department of Pharmacology, East Lansing, (Michigan 48823, USA), 19 January 1973.

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A Difference in Creatine Uptake Between Pectoralis and Thigh Muscles of the Chicken[1]

We have previously described a specific, saturable process that serves to transport creatine into skeletal muscle[2,3]. We now describe a difference in creatine uptake between pectoralis and thigh muscles which suggests that membrane transport of creatine varies from one type of muscle to another.

Materials and methods. Newly hatched male chickens, hybrids of a cross between Hubbard hens and White Mountain roosters, were given a commercial diet[4] and water ad libitum until they were 15 days old, when their average weight was 186 g. Then 1-^{14}C-creatine (5.08 mC per mmole)[5] was injected rapidly into a brachial vein. In addition to receiving ^{14}C-creatine, some of the chickens also received 3 ml or less of 0.154 M NaCl i.v., either alone or as a vehicle for non-radioactive creatine. Neither the NaCl solution nor the nonradioactive creatine adversely affected the appearance of the chickens, and the NaCl solution alone had no effect on the distribution of ^{14}C-creatine to muscle. At selected time intervals after injection the chickens were decapitated, exsanguinated, and individual muscles were obtained for measurement of radioactivity. The muscles were homogenized in 10 to 20 volumes of distilled water using a glass homogenizer, protein was precipitated by adding enough trichloroacetic acid to achieve a final concentration of 5% (w/v); and an aliquot of the clear supernate was taken for measurement of radioactivity by liquid scintillation spectrometry. Using paper chromatography in complementary experi-

Table I. Concentrations of selected compounds in pectoralis and biceps femoris muscles

Compound	Pectoralis (µmoles/g wet weight)	Biceps femoris
Creatine, total	32 $\pm$ 3.0 (11)[a]	30 $\pm$ 5.8 (7)
Phosphocreatine	19.4 $\pm$ 4.5 (6)	10.4 $\pm$ 2.5 (7)
Inorganic phosphate	5.5 $\pm$ 2.7 (6)	5.9 $\pm$ 2.1 (7)
ATP	6.4 $\pm$ 1.0 (6)	4.0 $\pm$ 0.45 (7)
ADP	0.99 $\pm$ 0.32 (6)	0.69 $\pm$ 0.08 (7)

[a] $\pm$ Standard deviation. Number of animals is given in parentheses.

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Table II. Effect of nonradioactive creatine on ^{14}C-creatine distribution

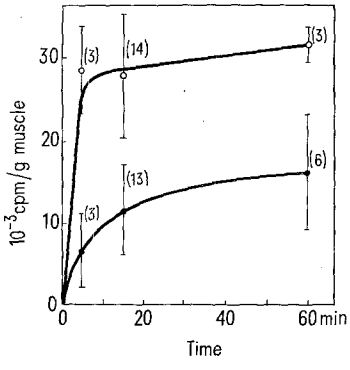
Specimen	Dose of creatine			
	0.055 mg (cpm/g wet weight)	2.9 mg	4.7 mg	26 mg
Pectoralis	11,600 ± 5,600 (14) [a]	19,000 ± 6,300 (4)	11,300 ± 3,500 (4)	9,300 ± 2,700 (4)
Biceps Femoris	28,000 ± 7,400 (13)	20,000 ± 2,600 (4)	12,300 ± 410 (4)	8,600 ± 570 (4)
Sartorius	29,000 ± 8,600 (9)	14,800 ± 2,900 (4)	12,900 ± 5,300 (4)	
Semitendinosus	22,000 ± 7,600 (7)	26,000 ± 3,300 (4)	14,700 ± 5,000 (4)	
Iliotibialis	25,000 ± 5,200 (7)	23,000 ± 2,500 (4)	17,000; 18,000 (2) [b]	
Plasma [c]	2,200 ± 720 (3)	8,800 ± 5,800 (4)	15,100 (1)	

[a] ± Standard deviation. Number of animals is given in parentheses. [b] Only 2 values were obtained; both are given. [c] Blood was collected in a heparinized container and centrifuged at 2500 rpm to obtain plasma; cpm/ml are given. Each chicken received one μCi of ^{14}C-creatine per 100 g body weight i.v. 15 min before it was killed; on the average this came to a dose of 0.055 mg of creatine. Some of the chickens received additional nonradioactive creatine i.v. immediately prior to the radioactive dose. The nonradioactive creatine was prepared at a concentration of 5.69 mg/ml in 0.154 *M* NaCl for the 2.9 and 5.7 mg doses and of 8.77 mg/ml for the 26 mg dose.

ments, we found that all of the radioactivity in these muscles migrated identically to authentic creatine.

Total creatine content of the muscles was measured by the method of ROSE, HELMER, and CHANUTIN[6] with minor modifications. The concentrations of inorganic phosphate, phosphocreatine, ATP, and ADP were determined on rapidly frozen muscles, obtained from anesthesized animals, by the automated chromatographic procedure described by JELLINEK, AMAKO, and WILLMAN[7,8].

Results and discussion. Curves depicting the uptake of a tracer dose of ^{14}C-creatine by pectoralis and biceps femoris muscles are shown in the Figure. Inspection of these curves reveals that the initial uptake of ^{14}C-creatine by biceps femoris muscles was approximately 4 times as great as that of pectoralis muscles. We found no evidence that the greater uptake of ^{14}C-creatine by biceps femoris muscle is due to trapping of the tracer as phosphocreatine (Table I). In fact phosphocreatine, ATP, and ADP were present in significantly smaller concentrations in biceps femoris muscles in comparison to pectoralis muscles ($P < 0.05$ by Students *t*-test[9]). The finding of a relatively low concentration of phosphocreatine in a red muscle confirms earlier reports by others[10,11].



Time course of uptake of ^{14}C-creatine by skeletal muscles. Each chicken received one μCi of ^{14}C-creatine per 100 g body weight i.v. at zero time. The mean of the cpm per g wet weight of muscle, the numbers of animals, and standard deviations are shown. o, biceps femoris muscle; ●, pectoralis muscle.

The distribution of ^{14}C-creatine was significantly altered by administering nonradioactive creatine as may be seen from the data presented in Table II. A total dose of 2.9 mg of creatine significantly increased the concentration of ^{14}C-creatine in pectoralis muscles ($P < 0.01$) while it either reduced or did not affect the concentration in various thigh muscles. With larger doses of nonradioactive creatine, the amounts of ^{14}C-creatine in thigh muscles and in pectoralis muscles was reduced significantly ($P < 0.05$) to values approximately equal to those achieved by pectoralis muscles of chickens given only the tracer dose of ^{14}C-creatine. The concentrations of radioactivity in plasma 15 min after injection also are given in Table II.

In the absence of evidence that the greater uptake of a tracer dose of ^{14}C-creatine by biceps femoris muscles is due to intracellular metabolism of creatine and since nonradioactive creatine would not be expected to alter bloodflow to muscle, we suggest that membrane transport of creatine varies from one type of muscle to another.

Zusammenfassung. Intravenös verabreichte Tracerdosis von ^{14}C-Kreatin führt beim Huhn zur viermal höheren Aufnahme im Biceps femoris als im Musculus pectoralis. Dieser muskuläre Unterschied fällt fort, wenn gleichzeitig grosse Mengen nicht-radioaktiven Kreatins gegeben werden.

C. D. FITCH, L. J. ROBBINS, M. JELLINEK and J. S. NELSON

Departments of Internal Medicine, Biochemistry, Surgery and Pathology, St. Louis University, 1402 S. Grand Boulevard, St. Louis, (Missouri 63104, USA), 9 February 1973.

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