Vitamin E Deficiency in the Monkey. VI. Metabolism of Creatine-1-C^{14}.* (29096)

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Creatinuria is a prominent feature of many muscle wasting diseases(1). Frequently the creatinuria is associated with reduced concentrations of muscle creatine and reduced urinary excretion of creatinine. The common association of these abnormalities with various muscle wasting diseases makes them nonspecific findings and thus reduces their diagnostic significance and obscures the important role that defects in creatine metabolism may have in diseases of muscle.

Over 90% of all the creatine in the body is in skeletal muscle(2). A large part of the creatine in muscle is phosphocreatine which is formed by the reversible phosphorylation of creatine. Because of the importance of phosphocreatine in energy metabolism of muscle, abnormalities of creatine metabolism in diseases of muscle deserve careful study.

Early studies with isotopic tracers showed that the end product of creatine metabolism in mammals is creatinine(3). The formation of creatinine *in vivo* is believed to be an irreversible nonenzymatic reaction that occurs mainly in skeletal muscle(2,4). Using this information it is possible to study the turnover of body creatine with isotopic tracers by measuring the isotope concentration of urinary creatinine at intervals after a dose of labeled creatine. Studies of this type indicate that the half-time of body creatine is approximately 20 days in mice(5), 29-36 days in rats(6,7), 42 days in man(8,9), and probably 40 days in dogs(10).

The effects of muscle wasting or of creatinuria on the turnover of body creatine have not been extensively studied. Studies of diseases having abnormalities of creatine metabolism might provide fundamental information concerning the normal metabolism of creatine in addition to defining further the abnormality in the disease studied. We have therefore determined the half-time of creatine in the normal rhesus monkey and in monkeys that had muscle wasting and creatinuria resulting from Vit. E deficiency.

Methods. The rhesus monkeys *(Macaca mulatta)*, the diets, and general handling of the animals used in this study have been described(11). Only animals receiving the purified creatine-free diet of Dinning and Day (12) were used. This diet contains lard and cod liver oil and is deficient in Vit. E. Control monkeys received DL-alpha-tocopherol in doses ranging from 20 mg per week initially to 240 mg per week during the last part of experiment. Tocopherol was given in 3 equal doses each week either orally as the acetate dissolved in ethanol or intramuscularly as the disodium salt of the phosphate ester dissolved in water. After 500 to 600 days of this diet, the unsupplemented animals used in the present study showed severe signs of Vit. E deficiency including anemia, histologic evidence of muscular dystrophy, and creatinuria(11). Since the unsupplemented monkeys developed the deficiency syndrome after varying intervals on the diet, a creatine turnover study was always done identically and simultaneously in a control monkey and a deficient one.

To study the turnover of body creatine each monkey received 10 μc of creatine-1-C^{14} (0.5 mc per mMole) per kilogram body weight either intraperitoneally or intravenously. After the creatine-C^{14} injection, muscle biopsies were obtained at one hour from one pair of monkeys and at 4 hours from the others. The muscle was used for histologic examination and for isolation of muscle creatine(13).

With care to eliminate contamination by food, urine was collected daily. The concentrations of creatine and creatinine of each urine sample were measured by the method

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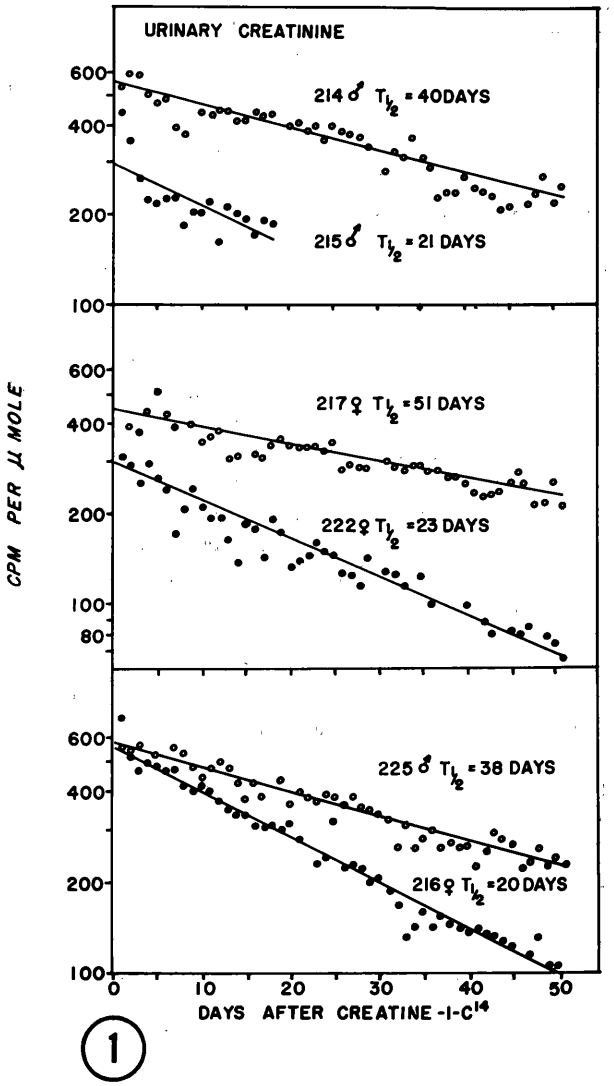
of Folin(14) with minor modifications. Creatine was then separated from creatinine by using Lloyd's reagent and the specific activities of the creatinine and creatine were determined by a method in which unlabeled carrier creatinine is used and creatinine zinc chloride is prepared for measurement of radioactivity(5). The radioactive samples were counted at infinite thickness in an automatic windowless gas-flow Geiger counter.

Results. The half-times of body creatine were 38, 40 and 51 days in the normal monkeys (Fig. 1). These values are similar to those of normal men(8,9) and greater than those of mice and rats(5-7). The longest half-time occurred in a post-pubertal female



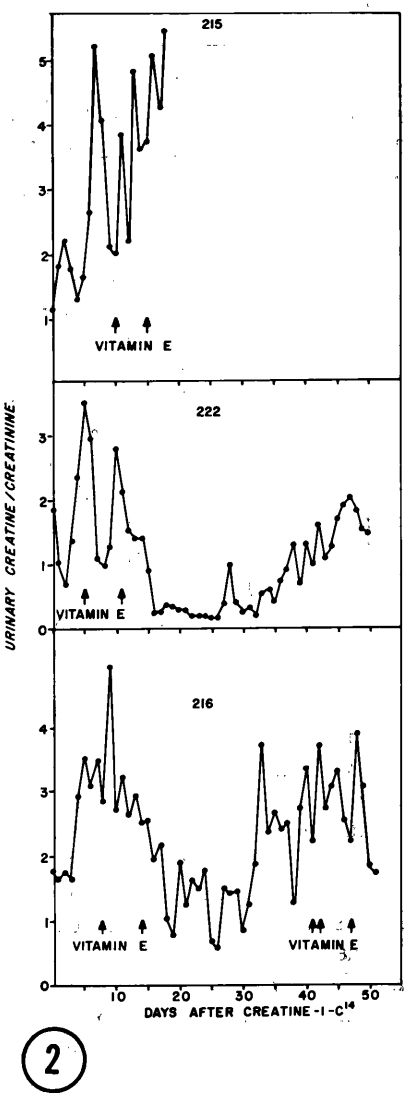


FIG. 1. Effect of vitamin E deficiency on decrease in specific activity of urinary creatinine. See Fig. 2 for days of Vit. E administration.

FIG. 2. Excretion of creatine by vitamin E-deficient monkeys. Ratio of urinary creatine concentration to concentration of creatinine is shown. Arrows mark days of Vit. E administration. Monkey 215 received the disodium salt of DL-alpha-tocopheryl phosphate intraperitoneally, 40 mg on day 10 and 60 mg on day 15. This animal died during day 18. Monkey 222 received DL-alpha-tocopheryl acetate dissolved in ethanol orally, 40 mg on day 5 and 150 mg on day 11. Monkey 216 received DL-alpha-tocopheryl acetate also, 100 mg on day 8, 100 mg on day 14, and 150 mg on day 41. On days 42 and 47 monkey 216 received 100 mg of the disodium salt of DL-alpha-tocopheryl phosphate parenterally.

TABLE I. Incorporation of Creatine-1-C^{14} into Muscle.

Monkey No.	Diet	Hours after creatine-C^{14}	Muscle creatine, cpm/μmole
214	Control	1	612
225	"	4	506
217	"	4	310
215	E-deficient	1	372
216	"	4	607
222	"	4	585

(monkey 217) and the shortest half-time occurred in a prepubertal male (monkey 225). With this small number of animals an effect of age or sex on the half-time of body creatine is not apparent, and if present, is not large.

In the vitamin E-deficient monkeys the half-times of body creatine were reduced to 20, 21 and 23 days. Each of these animals received Vit. E during the studies because they were extremely ill, but the apparent half-time of body creatine was unaffected. The best response to Vit. E therapy was obtained in monkey 222, in which the urinary creatine-to-creatinine ratio returned to normal and remained at normal levels for several days (Fig. 2).

During periods of significant creatinuria (creatine excretion equal to or greater than that of creatinine) the specific activity of creatine was measured. The creatine specific activity paralleled that of creatinine but was always significantly less. The average of the ratio of urinary creatine specific activity to creatinine specific activity was 0.87, 0.71, and 0.67 in monkeys 215, 216, and 222 respectively. As the control monkeys did not have significant creatinuria at any time measurements of their urinary creatine specific activity were not possible.

Table I shows the specific activity of muscle creatine at short time intervals after creatine-C^{14} injection. The specific activity of muscle creatine was not appreciably altered in the vitamin E-deficient monkeys. However, these animals had massive creatinuria (Fig. 2), presumably due to the high blood levels of creatine that are known to occur in vitamin E-deficient animals(15). High blood levels of creatine would lower the specific activity of creatine presented to the muscle of the vitamin E-deficient monkeys.

The results of these experiments in monkeys and of our earlier experiments in rats (16) are in conflict with the conclusions of Gerber and associates(17) from similar studies in rats. However, Gerber and associates did not measure muscle creatine specific activity until 24 hours after a dose of creatine-2-C^{14}; and, because of the fast turnover of serum creatine, this interval is too long to permit a reliable estimate of the initial uptake of creatine by muscle. Also, in the studies of muscle creatine turnover by these authors, different groups of animals were used for each point of the replacement curves. The groups were small and some of the points could only represent data from a single animal. As a further complicating factor the control and vitamin E-deficient rats used by Gerber and associates were reared on different diets. The control group received a stock diet until one week before the experiments were begun and the deficient rats received a purified diet. It seems likely that the defect in creatine metabolism due to Vit. E deficiency is the same in the rat as it is in the monkey.

Discussion. We have measured the half-time of total body creatine. Since most of the creatine in the body is present in skeletal muscle and because of studies(3,7) showing similar isotope contents of muscle creatine and urinary creatinine from rats given isotopically labeled creatine, it may be assumed that the half-time of muscle creatine is identical to that of body creatine. Using this assumption the reduced half-time of body creatine in vitamin E-deficient monkeys means a reduced half-time of muscle creatine.

A reduced half-time of muscle creatine indicates increased dilution of the creatine in muscle by newly synthesized creatine. This could result either from mixing of creatine across the muscle membrane or from regeneration of skeletal muscle containing newly synthesized creatine. A block in the entry of creatine into muscle, which has been suggested to explain the defect in creatine me-

tabolism in Vit. E deficiency(17), would not reduce the half-time of muscle creatine.

As further evidence against a block in the entry of creatine, the specific activities of creatine from the muscle biopsies of these monkeys (Table I) were not reduced by Vit. E deficiency. It is known that a large proportion of a dose of creatine is excreted in the urine when an animal has creatinuria (18). This would lead to reduced amounts of creatine-C^{14} available to skeletal muscle. Yet the specific activity of muscle creatine was as great in the deficient as in the control monkeys. Moreover, in vitamin E-deficient rats with mild creatinuria the specific activity of muscle creatine, shortly after creatine-1-C^{14} injection, is increased(16).

Regeneration of skeletal muscle in vitamin E-deficient animals(19) occurs and may explain in part our observations. Continued dilution of muscle creatine by the creatine of newly formed muscle is the most likely explanation for the failure of Vit. E therapy to alter the rate of turnover of body creatine even when creatine excretion returned to normal (Fig. 2). Unless newly formed muscle has a low creatine concentration, regeneration of muscle would not explain both the short half-time of body creatine and the reduced concentration of muscle creatine in vitamin E-deficient animals.

An impaired ability of skeletal muscle to retain creatine is the best single explanation for the combination of abnormalities of creatine metabolism in Vit. E deficiency. In support of a leak of creatine out of muscle is the high specific activity of urinary creatine at late time intervals. A leak of creatine from muscle has also been proposed to explain the abnormality of creatine metabolism in hereditary muscular dystrophy in mice (5) and in progressive muscular dystrophy in humans(20). Certain other muscle wasting diseases in humans(20) and muscle wasting due to transection of the spinal cord in the dog(10) do not reduce the half-time of body creatine. Thus the defect in creatine metabolism in vitamin E-deficient animals is not a nonspecific abnormality present in all types of muscular wasting.

A more precise understanding of the abnormality in creatine metabolism in nutritional and other types of muscular dystrophy must await new information about the mechanism maintaining the high concentration of creatine in muscle.

Summary. After creatine-1-C^{14} injection half-times of body creatine of 38, 40, and 51 days were found in 3 normal rhesus monkeys. Three vitamin E-deficient monkeys had shortened half-times of body creatine: 20, 21, and 23 days. This abnormality points to a defect in the ability of skeletal muscle to retain creatine as the explanation for the low concentration of creatine in muscle of vitamin E-deficient animals.

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