BODY-POTASSIUM CONCENTRATION AND RUBIDIUM METABOLISM DETERMINED BY WHOLE-BODY COUNTING IN DUCHENNE MUSCULAR DYSTROPHY AND ITS GENETIC CARRIER STATE

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Although potassium concentration is not a direct measure of body muscle mass, the whole-body ^{40}K measurement may prove to be a valuable way of estimating certain parameters of functional muscle mass in muscular dystrophy.

The present study provides data on body-potassium concentration and rubidium metabolism in patients with muscular dystrophy and in unaffected relatives measured by whole-body counting. Our efforts were directed to the study of the metabolic and etiologic relationship between childhood Duchenne muscular dystrophy and its genetic carrier state. We will present data concerning body-potassium concentration and the biological half-life of radioactive ^{86}Rb in patients with Duchenne muscular dystrophy and in genetic carriers. We will discuss the correlation between serum creatine phosphokinase activity, body-potassium concentration and the biological half-life of rubidium as a means of obtaining a more comprehensive method for detecting carriers of the disease.

METHODS

Body-potassium concentration was determined in 53 patients with neuromuscular diseases including 48 males and one female with the Duchenne form, two females with the limb-girdle form, one male and one female with the myotonic form and six with neurogenic atrophy (two males with amyotrophic lateral sclerosis, two males with Kugelberg-Welander's disease and two males with periodic paralysis). A total of 130 determinations were made with these patients and in 43 unaffected family members (15 fathers and 28 mothers and sisters). As controls in this study, 150 healthy males and 82 healthy females were measured.

A large plastic scintillator whole-body counter was used in this study because of its higher geometric efficiency and shorter counting time. Figure 1 shows a cutaway view of the system. Four large plastic scintillator units, each consisting of a 50 × 50 × 15-cm block, are placed 30 cm above the surface of a Lucite counting bed and four additional units are placed 10 cm below the bed. Scintillations in each unit are viewed by four 5-in. diameter photo-

Received July 10, 1967; revision accepted April 10, 1968.

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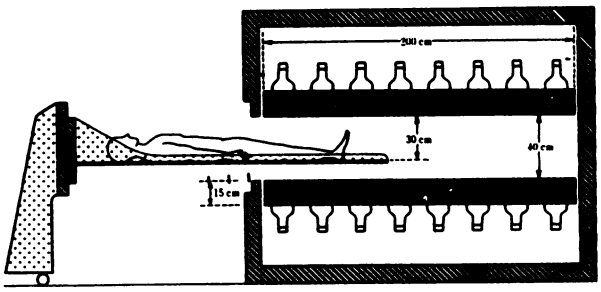

FIG. 1. Cutaway view of NIRS whole-body counter.

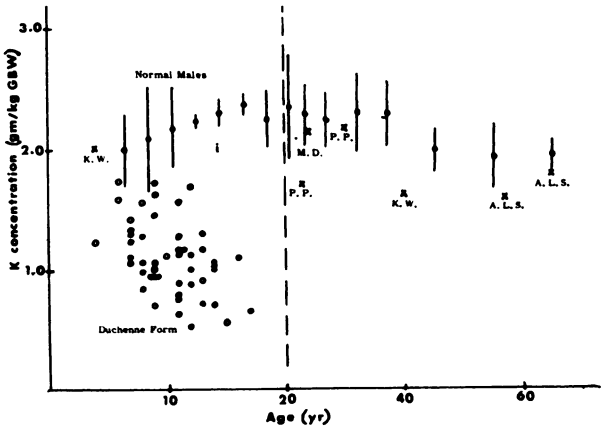

FIG. 2. Distribution of total-body potassium concentration as function of age for males.

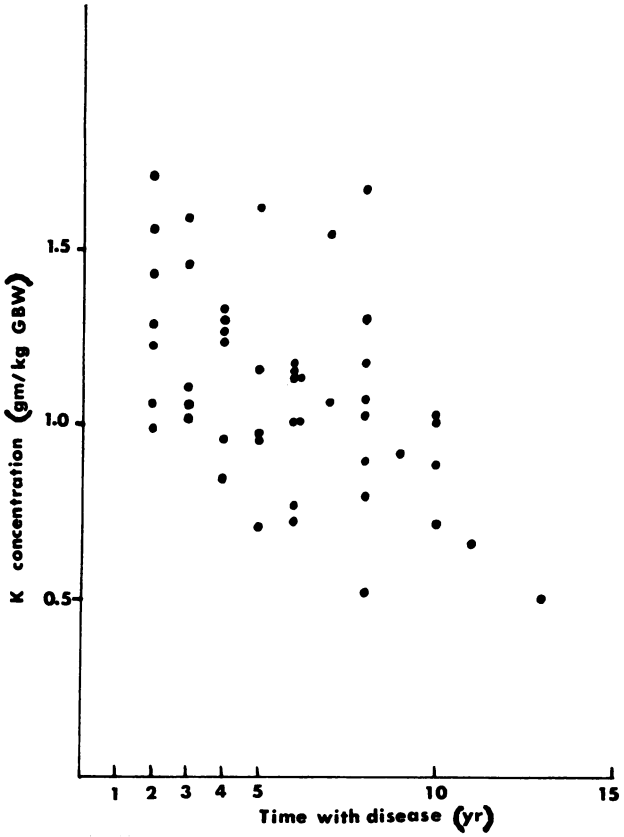

FIG. 3. Body-potassium concentration in patients with Duchenne muscular dystrophy plotted against duration of disease.

multiplier tubes. The combined output from the units was taken from a cathode-follower, mixed and then passed to a linear amplifier. The amplifier output was fed into three single-channel analyzers with associated scalers. Two single-channel analyzers selected the pulses for the potassium peak. One channel was set to select the upper portion and the other the lower portion of the peak. The sum of the pulses selected by the two analyzers was attributed to potassium. The ratio of the counting rates gave information about electronic adjustment and interfering radioactive contamination in the body. Another analyzer provided for the simultaneous measurement of ^{137}Cs radioactivity. The design and operating characteristics of this counter have been described in detail elsewhere (*1–3*).

The counter was calibrated using plastic body phantoms with a wide variety of lengths and weights filled with distilled water containing an exact amount of pure potassium chloride; it was also calibrated by administering a known amount of ^{42}K to humans. The calibration constants showed a linear relationship with the subject's weight. Correction was made for the different weights of each person. Instrument sensitivity was checked regularly by counting KCl and ^{137}Cs line sources.

Fifteen-minute counts were made on each subject and 1-hr background counts were measured periodically throughout the day. The detection efficiency of this counter was such that ^{40}K radioactivity in adult males could be determined with a statistical accuracy of ± 1.5% with a 15-min counting time. All data analysis was performed on a Burroughs-5500 digital computer.

Rubidium biological half-life. ^{86}Rb is not a precise tracer of potassium. However, because of its long physical half-life, it may provide some information about long-term potassium metabolism that could not be obtained by using ^{42}K.

An investigation of the possible use of $^{86}RbCl$ for long-term whole-body retention was performed in six male patients with Duchenne muscular dystrophy, and in nine carriers including seven possible, one probable and one definite carrier (*4*) and in two normal subjects.

Tracer doses of 3.8–4.1 μCi of ^{86}Rb were administered orally, and whole-body counting using the whole-body counter described above was performed 3 hr after ingestion and then once a month for 6 months.

Serum creatine phosphokinase (CPK) activity. The serum CPK activity of each carrier was determined by the "inorganic phosphate method" (*5–6*).

RESULTS

Figure 2 shows the distribution of body-potassium concentration (gm/kg of gross body weight) as a

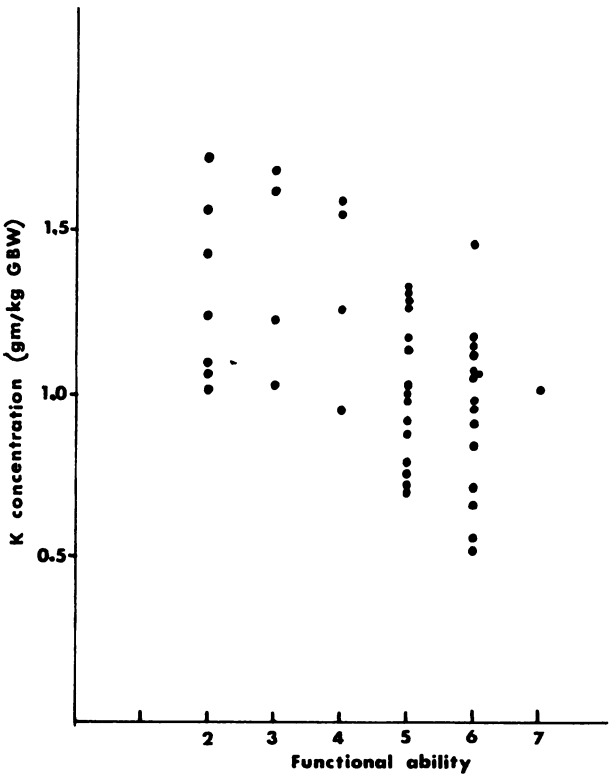

FIG. 4. Body-potassium concentration in patients with Duchenne muscular dystrophy plotted against stage of functional ability classified by Rusk and Deaver (*7*).

function of age for males. Normal males show increased body-potassium concentration as they mature, and the concentration tends to decrease with advancing age. In patients body-potassium concentration was within the normal range in the early stage of the disease, but it gradually decreased as the muscle wasting advanced. The decrease in body-potassium concentration was related to duration of the disease and functional disability of the patients, as Figs. 3 and 4 show.

Low potassium concentration was also demonstrated in females with limb-girdle muscular dystrophy, as Fig. 5 shows. Of eight patients with myotonic dystrophy or neurogenic atrophy, only one with periodic paralysis and one with Kugelberg-Welander's disease had decreased body-potassium concentration. The others were borderline or normal.

Figure 6 shows that 17 patients with Duchenne muscular dystrophy had a decrease not only in total body-potassium content but also in body potassium concentration during a period of about 1 year.

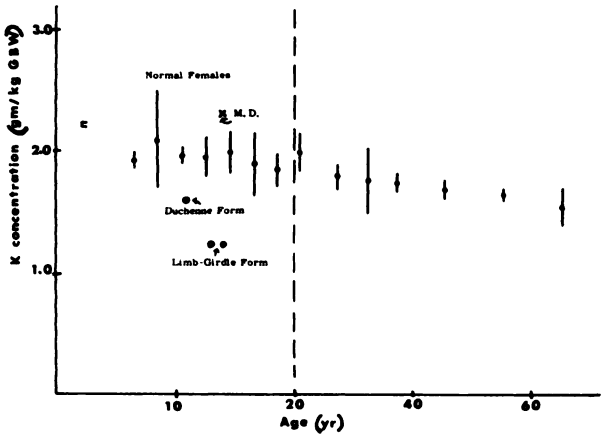

FIG. 5. Distribution of total-body potassium concentration as function of age for females.

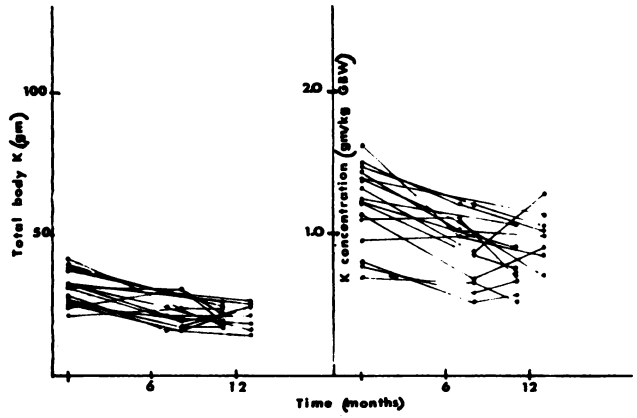

FIG. 6. At left, change of total-body potassium in Duchenne dystrophy patients during 1 year. At right, change of body-potassium concentration in Duchenne dystrophy patients during 1 year.

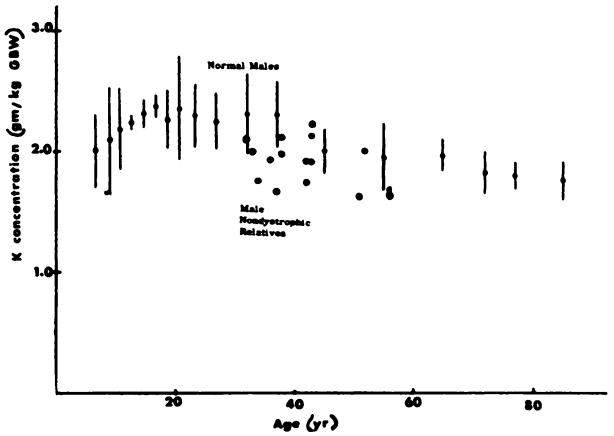

FIG. 7. Distribution of total-body potassium concentration in male nondystrophic relatives.

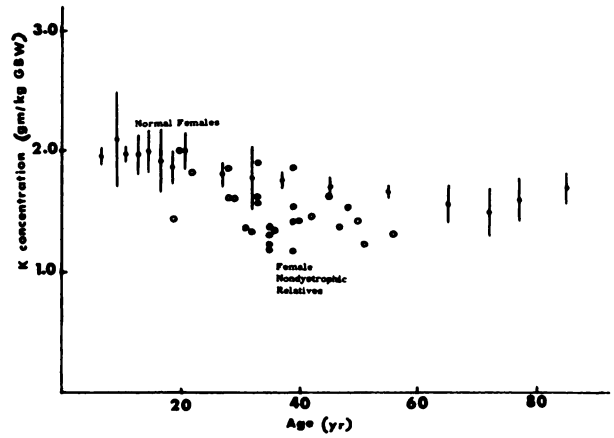

FIG. 8. Distribution of total-body potassium concentration in female nondystrophic relatives.

Fifteen fathers and 28 mothers and sisters, 20 of whom were known carriers, were measured for body-potassium concentration. They had no history of muscular weakness. (Obese subjects were excluded from these measurements.) As Figs. 7 and 8 show, remarkably reduced body-potassium concentrations were observed in about half of 28 female relatives, while slightly reduced concentrations were observed in only four of 15 male parents.

Correlation of serum CPK activity and body-potassium concentration is illustrated in Fig. 9. Among 20 carriers eight (40%) showed abnormal values both in serum CPK and body-potassium concentration, whereas five (25%) showed normal values. It is interesting to note that only one carrier showed normal body-potassium concentration with increased serum CPK, but six cases (30%) were found to have decreased body-potassium concentration, with normal CPK. In Fig. 10, body potassium

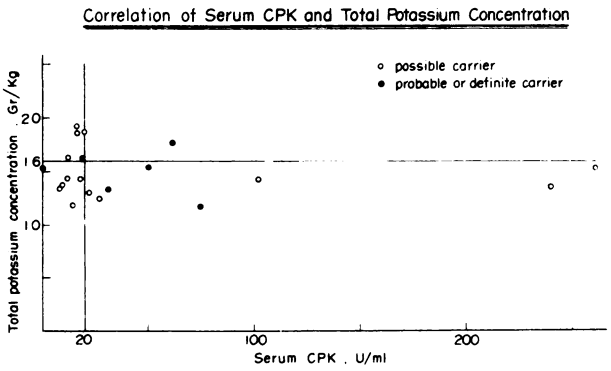

FIG. 9. Correlation of serum CPK and total potassium concentration.

concentration in relation to genetic carrier types are shown. Ten of 14 possible (70%) carriers showed decreased body potassium concentration. All of three probable carriers were found to have decreased body potassium concentration, but two of three cases of definite carriers showed normal body-potassium concentration. It should be noted that increased serum CPK was observed only in 32% of possible carriers, but the body-potassium concentration was lower in 70% of them.

Figure 11 shows long-term whole-body retention of ^{86}RbCl. The log percent retention decreased almost linearly with the lapse of time, showing a single exponential. The biological half-life of rubidium in patients with Duchenne muscular dystrophy was much shorter than in the normal subjects. It should be particularly mentioned that the biological half-life of nine carriers was found to lie between normal subjects and patients with Duchenne muscular dystrophy. In Table 1 the biological half-life of rubidium in these nine carriers is listed together with their serum CPK activities and body-potassium concentration.

DISCUSSION

This report emphasizes the importance of estimating body-potassium concentration and of determining long-term metabolism of ^{86}Rb by whole-body counting to assess Duchenne muscular dystrophy and the genetic carrier state.

Determination of total-body potassium in patients with progressive muscular dystrophy using a whole-body counter was first carried out by Blahd *et al* (*8–11*) and by Kossmann *et al* (*12*). Blahd observed decreased total-body potassium concentration in the preclinical or intial stages of muscular dystrophy. Additional evaluations of very young subjects before the stage of disability may be needed to establish whether or not potassium depletion precedes the clinical stage of the disease.

The decreased body-potassium concentration in patients is usually considered to be associated with the loss of functional muscle mass. Body-potassium concentration, however, depends not only on muscle bulk, but also on intracellular potassium concentration in affected muscle cells (*13–15*). Kossmann reported that the total-body potassium was a reflection of lean-body mass which was composed largely of potassium-rich muscle and that the potassium deficiency in muscular dystrophy was only a consequence of the wasting of dystrophic muscle rather than a reflection of a fundamental etiologic factor.

Blahd *et al* (*16,17*) reported that the ratio of total-body potassium to intracellular water derived by subtraction of extracellular water from total-body water was not significantly different in dystrophic patients and normal subjects. Their results indicate that dystrophic patients have normal intracellular potassium concentration and support the concept that the low levels of body-potassium concentration observed in dystrophic patients are in

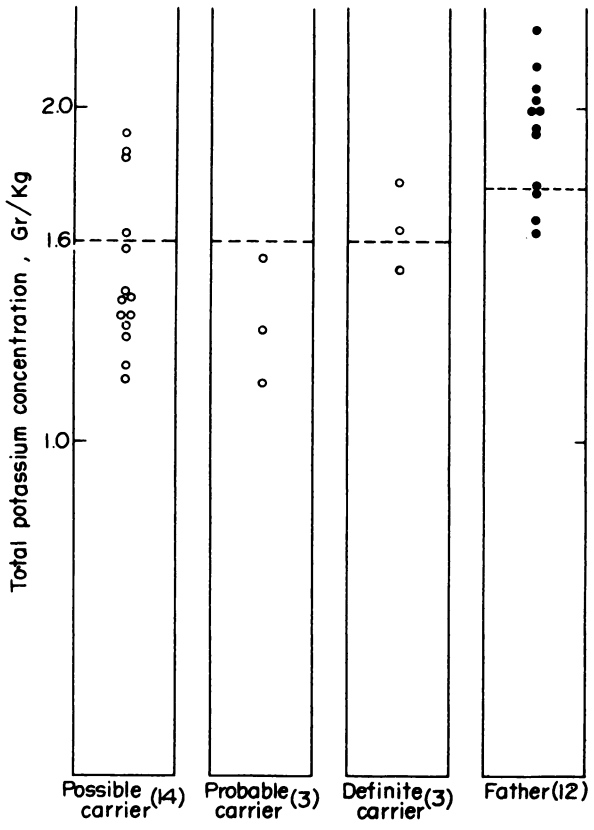

FIG. 10. Total-body potassium concentration of parents of patients with Duchenne muscular dystrophy. Types of genetic carriers were classified by Pearce's classification (4).

fact the result of replacement of muscle cells by collagenous tissue.

The mode of inheritance of Duchenne muscular dystrophy has been in much dispute. Although the disease seems to be a sex-linked hereditary disease, there are only a few published observations of total-body potassium measurements in relatives (*10,11, 16–19*). In 57 unaffected family members of patients with Duchenne or with limb-girdle muscular dystrophy, Blahd reported that unusually depressed body-potassium concentrations were noted in some nondystrophic parents and siblings of dystrophic patients, and such findings suggested a benign biochemical trait. Mays *et al* (*18*) reported that five healthy mothers of patients with Duchenne muscular dystrophy had normal potassium concentration. Unfortunately Blahd did not separate his subjects according to types of dystrophy and did not indicate which of the relatives were known carriers. The mothers in May's series were too few in number to draw conclusions.

Since the discovery of increased CPK in patients with Duchenne muscular dystrophy, this enzyme has been used not only for a differential diagnosis of the disease, but also for the detection of the genetic carriers. The increased serum CPK is supposed to be caused by the increased permeability of muscle-cell membrane.

TABLE 1. SERUM CPK, TOTAL-POTASSIUM CONCENTRATION AND BIOLOGICAL HALF-LIFE OF ^{86}RbCl OF 9 CARRIERS

Name	Classification of gene carriers	Serum CPK (U/ml)	K/GBW (gm/kg)	Biological half-life of ^{86}RbCl (days)
K.Y.	Possible	262.0 ↑	1.57 ↓	42.0 ↓
H.I.	Possible	1.4	2.20	39.5 ↓
Y.H.	Possible	18.4	1.49 ↓	35.0 ↓ ↓
U.H.	Possible	8.7	1.30 ↓	37.5 ↓ ↓
H.N.	Possible	7.6	1.34 ↓	44.0 ↓
Y.I.	Possible	19.9	1.86	35.0 ↓ ↓
S.U.	Possible	16.0	1.92	42.0 ↓
Y.H.	Probable	73.3 ↑	1.17 ↓	42.0 ↓
T.K.	Definite	0.9	1.53 ↓	30.0 ↓ ↓
Control (2 cases)		0–20	>1.6	51–66

Arrow and double arrow show the grade of abnormality.

The mothers of patients with Duchenne muscular dystrophy were classified into three types—possible, probable and definite carriers—following Pearce's classification (*4*). A possible carrier is a woman with one affected son and no other affected male relatives. A probable carrier is a woman with two or more affected sons but no male siblings or uncles affected. A definite carrier is a woman with an affected son and either a brother or a maternal uncle with the disease.

Thirty percent of all carrier types showed decreased body-potassium concentration but normal serum CPK. How can we explain the decreased body-potassium concentration in carriers in spite of their normal serum CPK? The normal serum CPK activity in some carriers may be explained on the basis that the dystrophic muscle fibers, which may have been present during childhood, might have undergone destruction by this time leaving only a few abnormal muscle fibers which would not significantly increase serum enzyme levels. This explanation is supported by the finding that the serum enzyme levels are highest in the preclinical as well as the early stage of Duchenne muscular dystrophy and tend to fall gradually toward normal values as muscle wasting advances and the subject becomes confined to bed or wheel chair. On the other hand, body-potassium concentration which may remain within the normal range in the preclinical stage of the disease, falls gradually as the disease advances.

It is also possible to say that decreased body-potassium concentration and increased serum CPK are different manifestations of the disease, the former

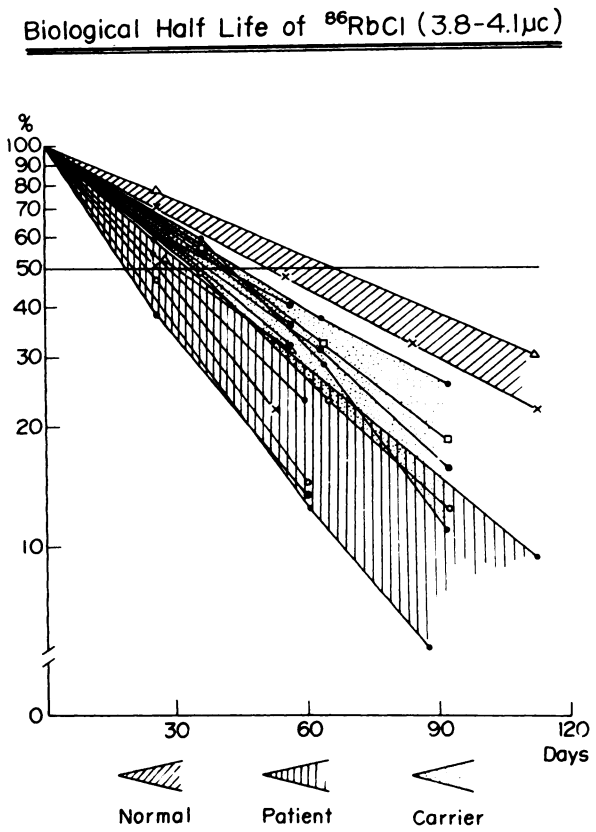

FIG. 11. Long-term whole body retention of ^{86}RbCl. Log percent retention decreases almost linearly with time, showing single exponential.

being correlated with decreased muscle mass and the latter with anamolous permeability of the muscle cell membrane. Therefore the body-potassium concentration is not a substitute for serum CPK determinations. We believe it is possible to increase the rate of carrier detection by determining both serum CPK and body-potassium concentration.

The biological half-life of ^{86}Rb of the nine carriers tested was shortened and lies between normal and dystrophic patients. If it can be assumed that rubidium represents the biological behavior of potassium, the time course of the whole-body retention of ^{86}Rb might serve as an indicator of the long-term metabolism of potassium. The results obtained suggest that the turnover of potassium is accelerated in both patients and carriers. These results are in agreement with ^{137}Cs studies reported by Mays (*19*). Mays who calculated the equivalent biological half-time of ^{137}Cs from the ^{137}Cs body burden and the ^{137}Cs urinary excretion reported that in ten patients with Duchenne muscular dystrophy the average half-time was about ¼ of normal values established for the same ages in healthy children. Further study, however, should be performed to reveal the mechanisms by which rubidium and cesium whole-body retention are decreased in patients with Duchenne muscular dystrophy and genetic carriers. It should be borne in mind that although rubidium and cesium are similar to potassium, many aspects of their metabolism differ significantly from potassium metabolism.

Although the mechanism of the accelerated turnover rate of rubidium is not yet clarified, the determination of biological half-life of rubidium using whole-body counting might be a new tool for detecting muscular dystrophy carriers. It might be possible to further increase the rate of carrier detection by combining the three methods described above: serum CPK, body-potassium concentration and the biological half-life of rubidium.

SUMMARY

Body-potassium concentration and long-term rubidium whole-body retention have been measured by whole-body counting in patients with Duchenne muscular dystrophy and in genetic carriers.

In the patients and in some of the carriers, body-potassium concentration was significantly lower than that observed in normal subjects of similar ages. A shorter biological half-life of rubidium was also found. These results suggest that determination of body-potassium concentration and rubidium half-life using whole-body counting may assume a role in diagnosing progressive muscular dystrophy and might be valuable as an indicator of the genetic carrier state, possibly predicting the inheritance of the disease.

ACKNOWLEDGMENT

The authors are indebted to K. Nakao of the Third Department of Internal Medicine, University of Tokyo for his valuable criticism and M. Iida of the National Shimoshizu Hospital for his cooperation.

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