Creatine Metabolism in Skeletal Muscle V. An Intracellular Abnormality of Creatine Trapping in Dystrophic Muscle[1] (34183)

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The list of abnormalities involving creatine in dystrophic mice includes the following: (a) low creatine content of skeletal muscle (1–4), (b) high rate of entry of creatine into extensor digitorum longus muscles *in vitro* (5), (c) short turnover time of body and muscle creatine *in vivo* (3), (d) high serum creatine concentration (2), and (e) excessive creatinuria (1–3, 6, 7). As discussed in earlier papers (3, 5, 8, 9,) abnormalities b and c indicate normal or greater than normal rates of replacement of muscle creatine, thus implicating accelerated loss of creatine as the cause of the low creatine content of dystrophic muscle. Accelerated loss of creatine from muscle, where most of the creatine in the body is located, also would explain the remaining abnormalities in the list (9).

In the present study of creatine loss *in vitro* from extensor digitorum longus muscles of normal and of dystrophic mice, we ask whether the defect responsible for accelerated creatine loss involves primarily the muscle cell membrane or primarily the intracellular metabolism of creatine. Our observations indicate that the defect is in the intracellular metabolism of creatine.

Methods. Dystrophic and normal mice of Bar Harbor Strain 129 were used for these studies. They were 1–2 months old when obtained and they were fed a complete, purified diet (3) for at least 1 week prior to study. Both male and female mice were studied, and some of the normal mice were heterozygous for the dystrophic trait, but neither an effect of sex nor one of heterozygosity was detected. The extensor digitorum longus muscles of normal mice weighed 6.5 mg each on the average, and those from the smaller dystrophic mice weighed 3.4 mg each on the average although both groups of mice were of the same age. Other characteristics of extensor digitorum longus muscles from these mice may be found in an earlier report (5).

To study creatine loss, muscle creatine was labeled by giving mice parenteral injections of creatine-1-^{14}C (sp act, 2.62 or 5.08 mCi/mmole)[3] 1 hr or 1 week before they were killed. Then the movement of radioactivity from their isolated extensor digitorum longus muscles into a Krebs–Ringer bicarbonate incubation medium was measured. The experimental conditions are given in the legends to the figures. Following incubation, the muscles were blotted to remove excess fluid and homogenized in 2 ml of fresh Krebs–Ringer bicarbonate solution. These homogenates and the incubation media were assayed for radioactivity either with a continuous gas-flow Geiger tube or with a liquid scintillation spectrometer.

Results. Figures 1 and 2 show losses of radioactivity (percentage) from extensor digitorum longus muscles of mice given creatine-1-^{14}C intraperitoneally 1 hr beforehand (short-term ^{14}C-creatine). The use of percentages instead of absolute losses minimizes the effect of having unequal amounts of radioactivity in the muscles at the beginning of incubation. After short-term ^{14}C-creatine the

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[3] Purchased from New England Nuclear Corporation.

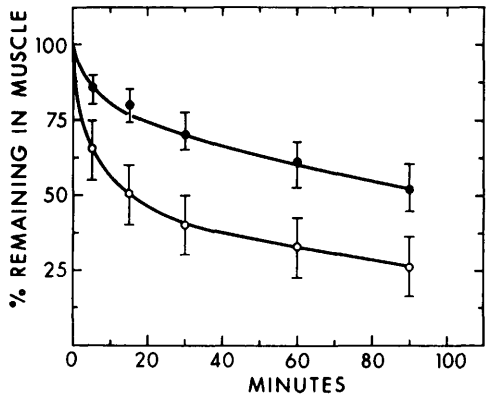

FIG. 1. Loss of short-term ^{14}C-creatine under aerobic conditions. Normal and dystrophic mice were each injected intraperitoneally with 2.0–3.75 μCi of creatine-1-^{14}C 1 hr before they were killed for these studies. Individual extensor digitorum longus muscles were incubated at 37° in 2 ml of Krebs–Ringer bicarbonate solution (pH 7.4) with an O_2:CO_2, 95:5, atmosphere. The muscles were transferred to fresh incubation media at each time interval. The percentage of the initial amount of radioactivity which remains in the muscle is shown on the ordinate. There were 16 normal (●); and 8 dystrophic (○) muscles. The means ± SD are shown.

average counting rates per extensor digitorum longus muscle were 646 cpm for normal and 547 cpm for dystrophic mice, and the corresponding plasma (extracellular) counting rates were 23 and 54 cpm/μl.

After short-term ^{14}C-creatine and under an oxygen atmosphere, part of the radioactivity was lost more rapidly from dystrophic than from normal muscles (Fig. 1). There was little difference between normal and dystrophic muscles under anaerobic conditions, however, because efflux of radioacitvity accelerated from the normal muscles (Fig. 2). In complementary studies using these same experimental conditions, nonradioactive creatine had no effect when added at a concentration of 1 m*M* to the incubation media of several muscles in each group.

Figures 3 and 4 show the losses of radioactivity from muscles of mice given creatine-1-^{14}C subcutaneously 1 week beforehand (long-term ^{14}C-creatine). The average counting rates per extensor digitorum longus muscle at the beginning of incubation were 786 cpm for normal and 446 cpm for dystrophic mice. Under an atmosphere of oxygen (Fig. 3), the efflux of radioactivity was small and there was little difference between the behavior of normal and of dystrophic muscles; but anaerobiosis impaired the ability of both groups of muscles to retain radioactivity from long-term ^{14}C-creatine (Fig. 4), and as the incubation progressed a difference between the groups became apparent. After approximately 60 min of incubation under nitrogen, the efflux of radioactivity from dystrophic muscles was less than from normal muscles. Thus, after 150 min of incubation under nitrogen, 31.5 ± 9.5% (mean ± SD) and 6.8 ± 4.5% of the radioacitivity remained in dystrophic and normal muscles, respectively.

Discussion. To explain accelerated loss of radioactivity from dystrophic muscle in experiments with short-term ^{14}C-creatine, there could be proposed a defect in the intracellular metabolism of creatine or a membrane defect, either of which would permit leakage of creatine. But since increased leakage through the membrane of dystrophic muscle cannot explain the less than normal anaerobic loss of radioactivity in experiments with long-term ^{14}C-creatine (Fig. 4), we may concentrate our attention on abnormal intracellular metabolism. Two types of intracellular defects are possible : defects in trapping creatine intracellularly or defects affecting the degradation of creatine.

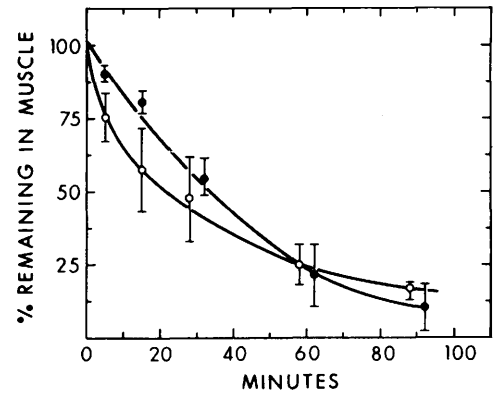

FIG. 2. Loss of short-term ^{14}C-creatine under anaerobic conditions. Except for an atmosphere of N_2:CO_2, 95:5, the details of this experiment and the meaning of the symbols are the same as those stated for Fig. 1. There were 6 muscles in each group.

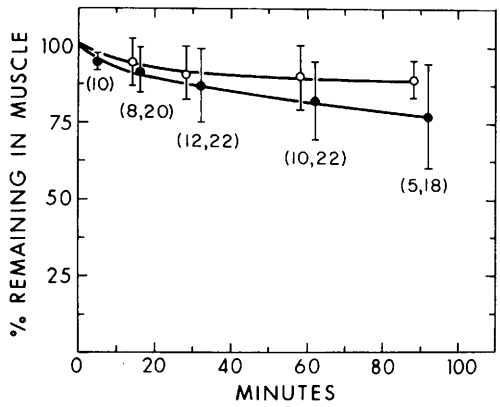

FIG. 3. Loss of long-term ^{14}C-creatine under aerobic conditions. Normal and dystrophic mice were each injected subcutaneously either with 0.1 or with 0.2 μCi of creatine-1-^{14}C/g of body weight; the dose was divided into two equal parts which were administered 24 hr apart. The mice were killed 1 week after the first injection of ^{14}C-creatine. Individual extensor digitorum longus muscles were incubated at 37° in 2 ml of Krebs–Ringer bicarbonate solution (pH 7.4) with an O_2:CO_2, 95:5, atmosphere. In most instances the muscles were transferred to fresh incubation media at each time interval, but the loss of radioactivity was the same regardless of whether or not the muscles were transferred to fresh media. The number of muscles studied at each time is given in parentheses. Means ± SD are shown; (●), normal muscles; (○), dystrophic muscles.

Abnormal degradation of creatine is not excluded by existing data, but it requires little discussion since there is no evidence of creatine degradation in skeletal muscle in the first place, except for slow nonenzymatic formation of creatinine (10, 11). In man and in the rat, searches for degradation products reveal creatinine to be the only significant end-product of creatine metabolism (12–14); and although the search for degradation products in the mouse is incomplete, creatinine is the only known end-product of creatine metabolism in this species too (3). Thus we may proceed to discuss the possibility of a defect in intracellular trapping of creatine.

A partial block in the intracellular conversion of creatine into a form inaccessible to the membrane would cause creatine with access to the membrane to accumulate and would permit the early rapid loss of short-term ^{14}C-creatine from dystrophic extensor digitorum longus muscles (Fig. 1). Conversely, a partial block in reconverting inaccessible creatine to the accessible form would explain retardation of loss (Fig. 4) in experiments with long-term ^{14}C-creatine. Finally, if the predominant block is in the conversion of accessible into inaccessible creatine, this would explain a low total content of creatine in muscle, a high blood creatine concentration, creatinuria, and short turnover times of muscle and body creatine.

From the foregoing considerations we now propose that, in skeletal muscle of dystrophic mice, there is impaired exchange between creatine with access to the membrane and creatine in a relatively inaccessible form. Whether or not inaccessible creatine is in fact phosphocreatine and whether or not the defect is in creatine phosphokinase (15, 16) are questions that cannot be answered yet. Inaccessible creatine might also be bound creatine (17, 18) or compartmentalized free creatine.

Summary. Radioactive creatine was given parenterally to normal and to dystrophic mice of Bar Harbor Strain 129 either 1 hr (short-term ^{14}C-creatine) or 1 week (long-term ^{14}C-creatine) before their extensor digitorum longus muscles were removed for study *in vitro*. After short-term ^{14}C-creatine and under aerobic conditions, part of the radioactivity was lost more rapidly from dystrophic

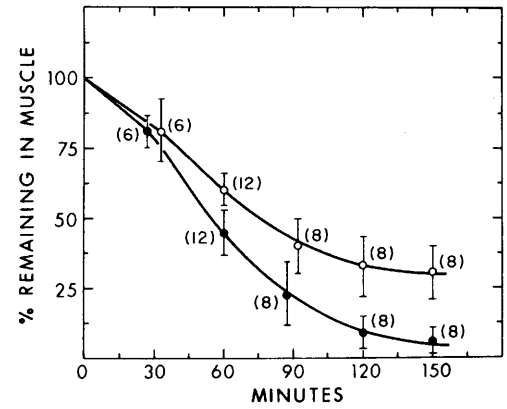

FIG. 4. Loss of long-term ^{14}C-creatine under anaerobic conditions. Except for an atmosphere of N_2:CO_2, 95:5, the details of this experiment and the meaning of the symbols are the same as those stated for Fig. 3.

muscles than from normal muscles; but, under anaerobic conditions, the losses of radioactivity from normal and dystrophic muscles were similar because the rate of loss from normal muscles accelerated. After long-term ^{14}C-creatine and under aerobic conditions, losses of radioactivity from both groups of muscles were small; but, under anaerobic conditions, part of the radioactivity was lost more slowly from dystrophic muscles than from normal muscles. These findings are evidence of an intracellular abnormality in the metabolism of creatine in dystrophic muscle.

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