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A STUDY OF ACETATE METABOLISM IN EXPERIMENTAL VIRUS-INDUCED DISEASE

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### Publication Date

1956-06-15

UNIVERSITY OF  
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Contract No. W-7405-eng-48

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ABSTRACT

The influence of experimental poliomyelitis on the oxidative metabolism of acetate by mice was investigated. The mice were given sodium acetate-2-C<sup>14</sup> and the excretion of breath C<sup>14</sup>O<sub>2</sub> was measured over a 7-hour period. Animals infected with poliomyelitis virus oxidized acetate at a lower rate and to a lesser extent initially following administration of substrate. After 7 hours, the difference in total C<sup>14</sup>O<sub>2</sub> excreted, although small, was significantly greater in mice manifesting paralytic symptoms of central nervous system involvement.

## A STUDY OF ACETATE METABOLISM IN EXPERIMENTAL VIRUS-INDUCED DISEASE

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The subject of acetate oxidation has been extensively investigated in a variety of organisms and under a variety of conditions.<sup>1, 2, 3</sup> One of the more satisfactory methods of studying such metabolism in the intact animal in vivo has been by the continuous recording of the  $C^{14}O_2$  excretion following injection of  $C^{14}$ -labeled acetate.<sup>4</sup> Such studies have been facilitated by the ready availability of specific labeled metabolites and by the development of convenient and reliable instrumentation.

There have been to date no data available concerning the effects of virus injection on acetate metabolism in experimental animals. This report describes a study carried out to determine the influence of poliomyelitis virus infection in mice on the oxidation of sodium acetate-2- $C^{14}$  to  $C^{14}O_2$ .

### MATERIALS AND METHODS

Webster strain white mice, 4 to 5 weeks of age, were inoculated intracerebrally with 0.03 ml of a suspension containing 10  $LD_{50}$  ( $LD_{50}$  = 50% lethal dose) of mouse-adapted MEF<sub>1</sub> (Type 2) poliomyelitis virus. In the period 4 to 8 days following inoculation, 20 infected mice were selected for metabolic study. Of these, 12 animals manifested distinct symptoms of central nervous system (CNS) involvement, marked by excessive irritability and limb paralysis, while 8 animals appeared healthy. These mice, along with seven normal controls, were injected intraperitoneally with 0.1 ml of a solution containing 1 ml of sodium acetate-2- $C^{14}$  with an activity of 5.0  $\mu$ c per mg. They were then immediately placed in an apparatus designed to measure respiratory radioactivity excretion.<sup>4</sup> This apparatus consisted of a simple flow system carrying the air from a small glass animal cage through an ionization chamber. The potential of the chamber was amplified by a vibrating-reed electrometer and charted on a continuous recording potentiometer. The  $C^{14}O_2$  respired was measured for 7 hours and the resulting curves analyzed to give the excretion of the  $C^{14}O_2$  as a function of time, in terms of both rate and cumulative excretion.

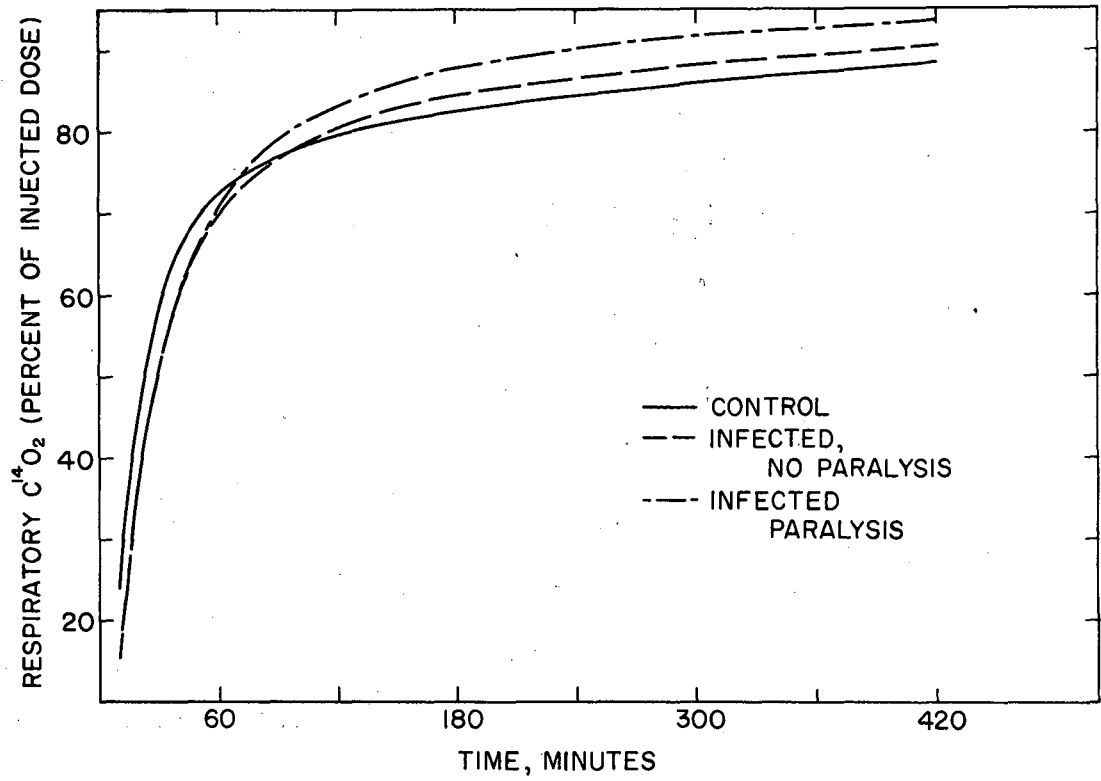
## RESULTS

The data obtained in this study are delineated in Figs. 1 and 2. The curves represent mean values for the carbon-14 respiration of the mice employed as described above. As can be noted from Fig. 1, the percent of the injected dose recovered as respired  $C^{14}O_2$  over a 7-hour period was highest (93.6%) in the paralytic animals, somewhat lower (90.4%) in the infected but healthy-appearing animals, and lowest (88.4%) in the normal control mice. With regard to the rates of excretion in Fig. 2, the peak rate was highest (3.8) in the case of the control animals, while those of both the paralyzed and healthy-appearing infected mice were about the same (2.7).

A statistical analysis was applied to test the significance of the enhanced cumulative  $C^{14}O_2$  excretion, on the one hand, and the depressed excretion rate, on the other, with infection with poliomyelitis virus. In each instance the test applied was a two-sided  $t$  test for testing the difference of the means of two normal populations with equal but unknown variances.<sup>5</sup> The  $F$  test was used in each case to check the equality of variances. On the basis of the statistical analyses performed, there was no evidence of significance in the difference in total amount of excreted  $C^{14}O_2$  between the healthy-appearing infected animals and the controls. However, the difference between paralyzed mice and the controls was significant at the 3% level. The differences observed in peak  $C^{14}O_2$  excretion rates were significant at the 1% level in both situations.

## DISCUSSION

The principal pathway for the oxidation of foodstuffs in animal tissues is presently considered to involve two major stages. In the first stage the substrate molecule undergoes a series of changes which ultimately result in the formation of either "active acetate" (acetyl coenzyme A) or an intermediate of the tricarboxylic acid cycle. In the second stage, a terminal oxidation occurs which yields much of the energy required by organisms; this latter stage is represented by the TCA cycle itself. Acetate administered to the animal may become reactive provided that coenzyme A and a source of high energy such as ATP are available. These two cofactors and the TCA cycle are common constituents of animal tissues.<sup>6</sup> Thus, in the presence of



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Fig. 1. Cumulative excretion of  $C^{14}O_2$  in normal and poliomyelitis-virus-infected mice following administration of sodium acetate-2- $C^{14}$ .



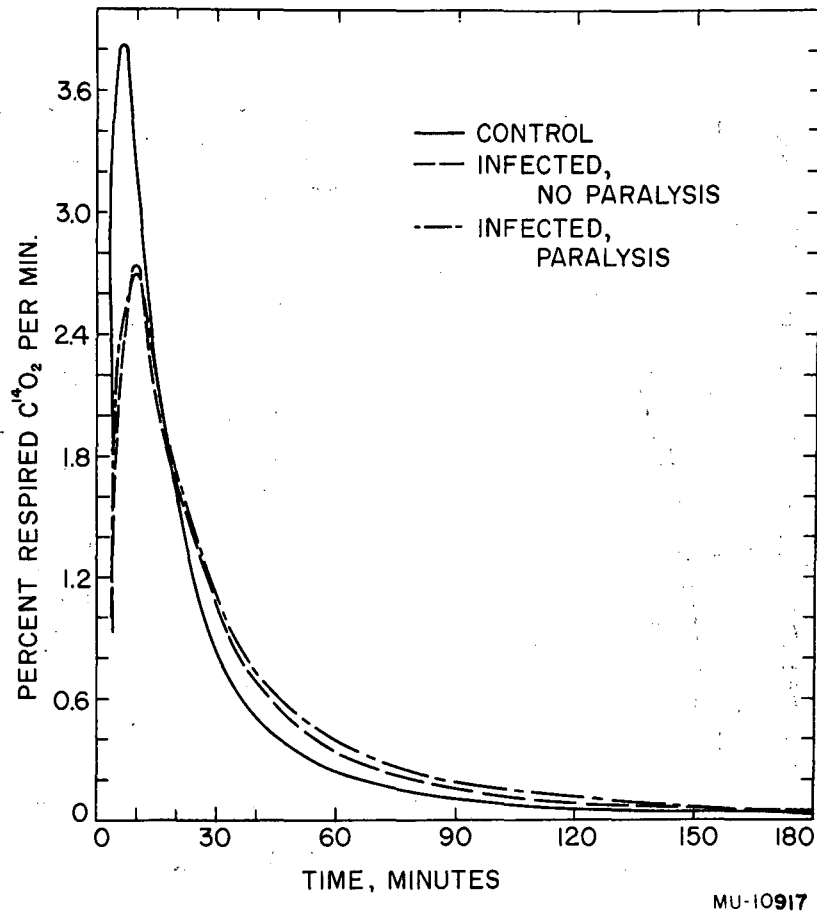
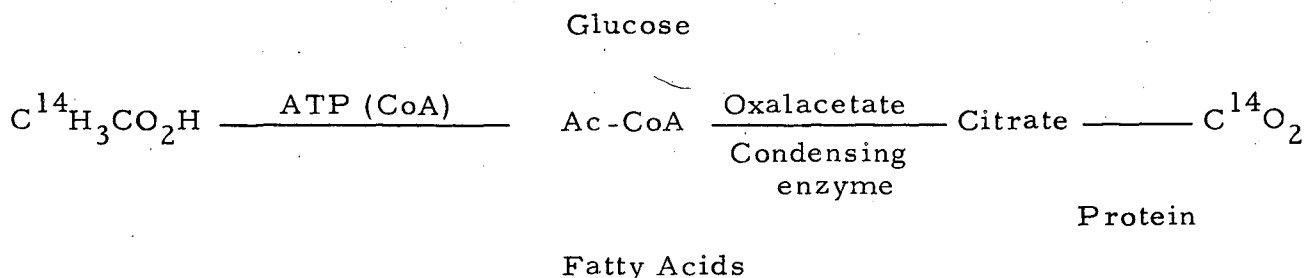


Fig. 2. Rate of excretion of C<sup>14</sup>O<sub>2</sub> in normal and poliomyelitis-virus-infected mice following administration of sodium acetate-2-C<sup>14</sup>.

these cofactors and other appropriate enzymes, the injected acetate might be expected to be actively metabolized in the animal tissues following any of the several pathways.<sup>7</sup> For the purposes of the study herein reported in which respiratory  $C^{14}O_2$  was determined, the metabolism of the two-carbon moiety may be schematically outlined as follows:



The lower rate of the labeled-acetate oxidation to  $CO_2$  by paralyzed mice in the first 6 to 10 minutes following substrate administration might be attributed to an initial lower body pool of the necessary cofactors to activate acetate, or to a possible shift in the state of dynamic equilibrium in the direction of diminished availability of oxalacetate or condensing enzyme. In about 20 minutes the rate of  $C^{14}O_2$  excretion by paralyzed mice was the same as that by normal mice. Thereafter the oxidation to  $CO_2$  of the label proceeded at a slightly higher rate, so that the difference in total  $C^{14}O_2$  respired after 7 hours, although small, was significantly greater for paralyzed mice. An increased rate of  $C^{14}O_2$  excretion in the paralyzed mice may represent an increase in acetate oxidation rate, but this can only be answered for certain if the information is coordinated with acetate body-pool size measurements. It may be that acetate activation itself continued at a greater rate in the normal mice, but that some of the "active acetate" was then employed in acetylation reactions or in fatty acid synthesis and (or) assimilation by way of tricarboxylic acid cycle intermediates, so that a portion of the acetate metabolized did not appear as  $C^{14}O_2$ .

In contrast to acetate, the extent of glucose oxidation was observed to decrease with poliomyelitis infection.<sup>8</sup> In mice with comparable paralysis, 63% of the uniformly labeled glucose- $C^{14}$  dose was respired as  $C^{14}O_2$  as compared with 70% for the controls. However, the depression in the peak rates of substrate oxidation with virus infection was of a similar order with both acetate and glucose. Both the glucose and acetate oxidation data are most interesting, not because of the magnitude of the changes observed

between the normal and paralytic animals, but because of the lack of any large effect. From numerous experiments carried out in this laboratory it appears that, up to the point of moribundity, as long as the animal is alive the intermediary metabolic pathways proceed at an approximately normal rate. This surprising effect has also been observed by the authors in other pathological situations, such as in advanced cancer and starvation.

While gross respiratory studies such as these require further corroboration by tissue and in vitro enzyme studies, they do indicate significant differences and provide the essential insight upon which to base subsequent studies.

### SUMMARY

The influence of experimental poliomyelitis on the oxidative metabolism of acetate by mice was investigated. The mice were given sodium acetate-2-C<sup>14</sup> and the excretion of breath C<sup>14</sup>O<sub>2</sub> was measured over a 7-hour period. Animals infected with poliomyelitis virus oxidized acetate at a one-third lower peak rate initially following administration of substrate. After 7 hours, about 5% more total C<sup>14</sup>O<sub>2</sub> was excreted by mice manifesting paralytic symptoms of central nervous system involvement than by normal mice. That this difference is no greater is particularly interesting, considering the drastic changes in physiological conditions associated with paralytic poliomyelitis.

This work was done under the auspices of the U.S. Atomic Energy Commission and of the National Foundation for Infantile Paralysis and the American Cancer Society.

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