The Suppression of Mouse Spontaneous Locomotor Activity by the Ingestion of Deuterium Oxide

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Summary. The spontaneous locomotor activity of the laboratory mouse is significantly depressed by the ingestion of deuterium oxide. The response, which is reversible, is a rectilinear one with up to 70% reduction in activity with the administration of 25% heavy water.

The circadian rhythm in spontaneous locomotor activity in CF-1 mice has been described in detail previously[2], as has been the effect of deuterium-oxide ingestion on: the period length of the rhythm in constant conditions[3], the phase relationship maintained with a light-dark cycle, and the loss of entrainment[4–6]. The study reported in this paper describes the deuterium caused depression of locomotor activity in this animal.

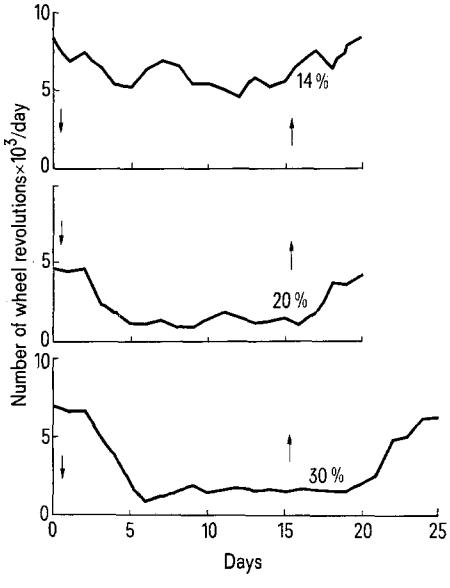

Fig. 1. Representative individual responses of mice to deuterium ingestion. The Day 0 value is the average daily activity for the 10 days preceding the introduction of deuterium. The falling arrows signify the beginning of D_2O consumption; the ascending arrows, the return of the mice to plain water.

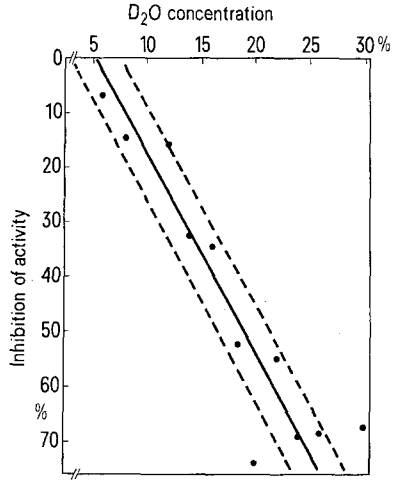

Fig. 2. The suppression of spontaneous locomotor activity by the ingestion of various concentrations of deuterium oxide. The curve was fitted to the data by the method of least squares. The Y intercept is 5.44, the slope is 0.278, and the Pearson coefficient of linear correlation is 0.918. The dashed lines on either side of the solid curve indicate ± one standard error of the estimate (Sy.x = 2.82).

Materials. The common laboratory mouse (*Mus musculus*) was maintained individually in self-cleaning cages to which an activity wheel was attached (illustrated in[7]). A cam attached to the outer margin of the running wheel caused the contacts of a microswitch to close with each revolution of the wheel. This, in turn, activated a digital counter which recorded the rotation. At the same time each day, the number of revolutions accumulated on the counter was recorded and the counter reset.

The mice were fed ad libitum on Purina Lab Chow, and the food and water reservoirs in each cage contained a 10–14 day supply, thus permitting the need for only occasional interruptions of the experimental conditions for reasons of husbandry. Either tap water, or various amounts of deuterium oxide (New England Nuclear) mixed with tap water, were offered to the mice.

The lighting conditions for the mice during storage and through all of the experimentation consisted of 12 h of illumination (11 foot candles from 'cool white' fluorescent bulbs) alternating with 12 h of darkness. The temperature was held at 20 ± 1 °C. in constant temperature rooms.

Procedure. The mice were fed tap water for approximately 2 weeks and then switched to various concentrations of D_2O ranging between 6 and 30%, in roughly 2% increments, for 11-15 days. In one experimental series, the amount of heavy water consumed was also measured. During the final 2 weeks of each series, the mice were returned to ordinary tap water again. 46 male mice were used in the study.

Results. When drinking deuterium and water combinations, all of the mice tended to consume about the same amount of liquid each day: 12.5–16 ml. This finding does not agree with that of KATZ et al.[8], who reported that the amount of fluid intake decreased as the concentration of deuterium in the drinking water increased.

Decrease in the amount of daily activity began to occur only about 2–3 days after the onset of heavy water ingestion (Figure 1). The activity then continued to decrease over the next few days to a level which remained essentially constant for each concentration of heavy water offered during the remainder of the time it was ingested. The values from this latter state were used in the construction of Figure 2, where it is seen that there is a linear relationship between activity depression and D_2O concentration.

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Upon removal of D_2O from the drinking reservoir and the restoration of tap water, there was a gradual recovery over a period of 3–5 days back to the pretreatment level of activity (Figure 1).

Discussion. It is known from the work of KATZ et al.[9], who performed serial analyses of urine from mice maintained on 15–30% heavy water solutions, that it takes from 6–8 days after the initiation of D_2O drinking, for a constant level of deuterium to be reached in their body fluids. However, at the end of the first 24 h, 45–60% of this equilibrium value has been attained. Furthermore, for a constant level of deuterium to be incorporated into the tissues by biosynthesis and exchange with hydrogen, requires another 2 weeks. In the study we report here, it was found that maximum inhibition of activity was reached roughly at the time that full deuteration of the interstitium was completed. Paradoxically, no further inhibition occurred in spite of the fact that deuterium build-up in the mice continued at a steady rate, especially at the higher concentrations. We have no ready answer for this finding.

To the best of our knowledge, this is the first published account of the inhibitory effect of deuterium on spontaneous locomotor activity (however, in our laboratory, PALMER and GOODENOUGH have found a similar response in the amount of perch hopping of the African waxbill, *Estrilda*). In one sense, it is an unexpected finding since it is known that at a slightly higher concentration, 40% (which is toxic), the metabolic rates and body temperatures of mice are significantly increased, and they become hyperactive[10]. Additionally, the decrease we found cannot be attributed to being just an overt sign of a general malaise caused by deuterium in the animal, because it is known that mice can live on D_2O concentrations as high as 30% for as long as 10 months without the appearance of any adverse effects in their health[11].

GARBY and NORDQUIST[12] have demonstrated in vertebrates that there is a 20% reduction in the conduction velocity of nerve fibres immersed in 99% D_2O, and KAMINER[13,14] has found that the force of contraction of frog and rabbit skeletal muscle is decreased significantly by deuterium. Both of these findings, if they hold for mouse nerve and muscle tissues also, might be expected to play an important role in decreasing the levels of locomotor activity in deuterium-treated animals. At present, however, we have no evidence to back this speculation.

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Mechanisms of Increased α_1-Fetoprotein Production in Hepatic Injury

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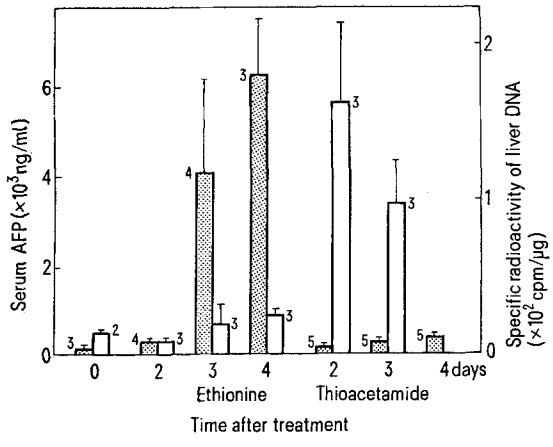
Summary. The increased production of α_1-fetoprotein in injured liver is primarily associated with hepatic injury and not with liver cell regeneration.

The ambiguity about the mechanisms of increased α_1-fetoprotein (AFP) production, either by hepatocellular regeneration or injury in damaged liver, may arise from the fact that hepatotoxins previously tested[1], such as carbon tetrachloride, produce liver cell necrosis as well as regeneration during the process of liver injury. In the present study, two hepatic poisons, ethionine and thioacetamide, were used to produce one or the other of these hepatic lesions in an attempt to investigate the association of increased level of serum AFP and stimulated DNA synthesis in liver.



Serum AFP concentrations (shaded bar) and incorporations of ^{3}H-thymidine into liver DNA (open bar) after single injections of ethionine and thioacetamide to male rats. Animals were treated as described in the text. Values along the bars are the number of animals per each group. The vertical line on each bar indicates the SEM.

Materials and methods. A single i.p. injection of thioacetamide (5 mg in saline/100 g body weight) and DL-ethionine (100 mg in saline/100 g body wt.) was given to overnight fasted Sprague-Dawley rats. Animals were then fasted and given only water for 2 days. For more than 2 days of experimental periods, the rats were fed ad libitum on Laboratory Chow until sacrificed. Control rats receiving an equivalent amount of saline were treated similarly. All the treatments were designed so that the age of rats was 35 days after birth at the time of sacrifice. The incorporation of ^{3}H-thymidine into liver DNA, serum AFP concentration and hepatic glucose 6-phosphate dehydrogenase (G6PD) activity were determined as described previously[2,3]. Serum alanine aminotransferase (GPT) activity and liver triglyceride content were measured by the routine laboratory methods.

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