PAPER NO. 3

STUDYING THE EFFECTS OF EXERCISE DURING EXPOSURE TO INHALED POLLUTANTS USING ANIMAL EXPOSURE MODELS

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INTRODUCTION

Animals are used in toxicology studies to gain information about questions which cannot be approached with human subjects. Human volunteers may be inappropriate due to either the hazardous nature of the test substance, invasive procedures required by the experiment, or a need for large numbers or lifespan studies of test subjects. In these cases animals are used as models of human biology, and it is important that the route of entry and conditions of exposures approximate as best as possible the conditions of real human exposures.

Most animal inhalation studies are exposures at rest; however, actual human exposure situations usually involve some level of activity which can be strenuous in both recreational and occupational settings. Intensity of activity is characterized by metabolic rate, usually heat production or oxygen consumption ($\dot{V}_{O2}$), and is conveniently expressed as a factorial increase over resting rate. Table 1 lists oxygen consumption rates associated with various human activities. Common activities require metabolic rates about 3 times resting rates, and a measure of average metabolic expenditure of daily life is about twice the cost of quiet rest. Maximum oxygen consumption of humans is up to 16 times resting. Metabolic gas exchange is supported by respiratory ventilation; thus, the respiratory system is designed to accomplish gas exchange at maximal levels of activity. At rest only a small part of the functional reserve of the respiratory system is involved in ventilation and gas exchange.

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TABLE 1. METABOLIC COST OF VARIOUS ACTIVITIES, 80 Kg HUMAN

	$\dot{V}_{O2}$ (L/min)	Factor Increase Relative to Rest
Basal Metabolism	0.24	
Resting (sitting)	0.26	1.0
Walking (4 km/h)	0.82	3.2
Running (10 km/h)	2.9	11
Maximum ($\dot{V}_{O2}$)	4.2	16
Light Manual Labor		
(carpentry, painting, raking)	0.7-0.9	3.0-3.5
Heavy Manual Labor		
(digging, woodcutting)	1.4-1.8	5.4-6.9
Recreational Exercise		
(bicycling, tennis, basketball, dancing)	0.8-2.0	3.1-7.8
(cross country running)	2.1	8
Average Daily Metabolic Expenditure	0.45	1.7
Average Metabolic Expenditure During		
Waking Hours	0.50	2.1

Data based on Consolazio et al. (1963) and Schoeller and van Santen (1982).

During exercise several events can dramatically change the effects of inhaled toxic substances. Increased respiratory ventilation exposes an individual to a larger inhaled dose of an airborne compound, and the dose rate may be many fold greater than resting exposure. Biological effects may not be simply proportional to increased dose rate due to exercise ventilation. Greater depth of breathing, decreased contact time between inspired air and upper airways, and an increase in the fraction of air drawn through the mouth rather than the nose result in alteration of deposition efficiences of gases and particles in the respiratory tract and changes in the distribution of inhaled dose (Valberg et al., 1982; Saibene et al., 1978; Frank et al., 1969). Any protective breathing patterns operating at rest in response to irritant compounds may be overridden by the demands for increased gas exchange while exercising. Thus, the health risk of exposure during exercise may be considerably greater than predicted by simple proportion to inhaled dose. Accordingly, inhalation studies of air pollutant compounds with human subjects frequently used exercise exposures, and they have typically demonstrated greater responses during exposures at exercise than were observed at rest (DeLucia and Adams, 1977; Folinsbee et al.,

1977; Sheppard et al., 1981; Silverman et al., 1976; Bates et al., 1972). By comparison to the extensive work with human subjects, there have been relatively few studies of exercise effects in animal inhalation studies. The purpose of this presentation is to describe some of the ways in which exercising animals can be applied to inhalation toxicology.

ANIMAL EXPOSURE MODELS

Rodents have been the subjects of early exercise inhalation studies. Rats and mice exposed to ozone while exercising in a motor driven cage demonstrated increased toxic responses including reduction in lethal ozone concentration, increased susceptibility to bacterial infection, and increased lung tissue levels of reduced glutathione (Stokinger et al., 1956; Gardener et al., 1974; Fukase et al., 1978). Depression of spontaneous activity due to exposure to O_3, NO_2, and SO_2 was studied in mice by recording individual use of an exercise wheel (Stinson and Loosli, 1978). In a recent study of aerosol deposition, hamsters were exposed while running on a treadmill and exhibited increased retention of inhaled particles compared to animals exposed at rest (Harbison and Brain, 1983).

The purpose of performing exercise exposures of animals is to increase inhaled dose rate and hopefully change dose-distribution in a manner analogous to conditions of human exercise exposure. In the absence of specific information on dose distribution changes and relative tissue sensitivity between species, the most reasonable approach to similiarity of exercise conditions is to use equivalent factor increases in ventilation and metabolic gas exchange above resting rates. Exercise workload can be very simply quantified by running speed and grade (Wranne and Woodson, 1973); however, measurements of metabolic gas exchange are more representative of relative ventilation and more easily compared to other studies. Ideally, the inhalation toxicologist would like to know minute ventilation as the measure of dose. Unfortunately, $\dot{V}_E$ cannot presently be measured in freely running small mammals such as rodents. However $\dot{V}_{O2}$ and $\dot{V}_{CO2}$ can be measured by analysis of input and output gas fractions and flows (Harbison and Brain, 1983; Mautz et al., 1984). Ventilation is proportional to $\dot{V}_{O2}$ up to the anerobic threshold above which it increases more rapidly than $\dot{V}_{O2}$ and finally more rapidly than $\dot{V}_{CO2}$ (Wasserman et al., 1981). Moderate exercising exposures which can be extended for durations of hours will not exceed anaerobic threshold; however, it should be noted that inhalation of respiratory irritants could alter the relation between ventilation and gas exchange.

Rodents can be easily trained to exercise on treadmills or running wheels. A program of exercise training is usually necessary to acquaint the animals with the apparatus, and extensive training can serve to develop exercise stamina (Bedford et al., 1979; Patch and Brooks, 1980). Laboratory rats are the most frequent rodent subjects of exercise studies. Although laboratory rats will run on treadmills, their exercise capacities are limited by comparison to most other mammals. Most mammals can achieve a maximum $\dot{V}_{O2}$ about 10 times resting rate (Taylor et al., 1980), but laboratory rats have factorial metabolic scopes of only about 5 (Armstrong et al., 1983; Bedford et al., 1979; Mautz et al., 1984), and at high exercise levels, they exhaust quickly. My colleagues and I have found that with progressive training over a 3 day period, Sprague-Dawley rats are capable of sustaining running at 2 to 3 times resting $\dot{V}_{O2}$ for 4 hours in O_3 atmospheres up to 0.4 ppm. These exercise conditions are analogous to the metabolic increment and duration of human exposures at mild workloads (Table 1). Treadmills and running wheels may be enclosed to contain exposure atmospheres (Mautz et al., 1984; Harbison and Brain, 1983), or if they are sufficiently small they can be placed inside a larger chamber (Stokinger et al., 1956; Puente et al., 1958). Ganged wheels or multichannel treadmills permit simultaneous exposure of several animals. Treadmills offer the advantage of easier access to the running animals, effective exposure of the animals in a stream of air flowing down the runway, and the capacity for varying both speed and grade of running. The major advantage of rodents in exercise studies is that they are relatively inexpensive and can be exposed in large numbers. The principal limitation of rodents or other small mammals is the difficulty of measuring pulmonary function variables. With the exception of gas exchange, most pulmonary function measurements require some form of restraint or quiescent behavior. Blood samples can be drawn from exercising rats prepared with arterial and venous cannulas (Gleeson and Baldwin, 1981); however, most other techniques must be reserved for post exercise tests.

Dogs have been extensively used in studies of exercise physiology, but have only recently been applied to exercise inhalation toxicology (Reischl et al., 1982; Mautz et al., 1983). Dogs offer distinct advantages over rodents for exercise physiology. They have a large aerobic scope, they can be easily trained to run on treadmills, and they can be trained to wear respiratory masks and other apparatus necessary for measurement of pulmonary function variables. Due to the large size of treadmills needed for dog exercise, inhalation exposures are most efficiently performed with a respiratory mask (Stavert et al., 1982 B). A disadvantage of dogs as study subjects is the expense

of maintaining and testing large animals. Extensive space and personnel must be devoted to housing and care, and due to limitations of resources, exercising exposures must usually be restricted to single subjects at a time. Another complication of exercising exposure of dogs is the panting ventilation used for thermoregulation. Under heat loads induced by exercise, dogs increase and preferentially direct ventilation to the tissues of the nose and mouth for evaporative cooling (Schmidt-Nielsen et al., 1970; Goldberg et al., 1980). Clearly, this distinctive thermoregulatory breathing pattern not present in humans is undesirable in a model animal species; however, by cooling dogs in a refrigerated treadmill, panting thermoregulation can be suppressed (Stavert et al., 1982A; Reischl et al., 1982).

RESULTS OF RECENT EXERCISING EXPOSURE EXPERIMENTS WITH RATS AND DOGS

Research by my colleagues and myself on exercise as an important modifier of toxic responses to inhaled air pollutants has focused on tissue damage to the lungs of rats and pulmonary function changes in dogs. Below I describe experiments we have performed exposing rats to 0.35 ppm O_3 and dogs to 10 ppm formaldehyde.

Exercising Rats Exposed to 0.35 ppm O_3

A treadmill inhalation system was constructed in which 10 rats were simultaneously exposed to O_3 and exercise effort was measured by average metabolic gas exchange (Mautz et al., 1984). Two days following exposure, the animals were sacrificed, the lungs were removed and fixed by airway perfusion with 10% neutral buffered formalin at 30 cm fluid pressure (McClure et al., 1982). Lung tissues were embedded in paraffin and sectioned at 6 μm and stained with hematoxylin and eosin. Focal parenchymal lesions induced by ozone exposure were quantified as percent of parenchymal cross section area involved. Two morphological types of lesions were identified: Type I - free cells in alveolar spaces with no apparent changes in septal walls. Control clean air exposed rats exhibited these features to a small degree (1-3% cross section area); however, the finding was categorized as a lesion because the incidence increased dramatically following exposure to O_3. Type II - Alveolar duct walls and alveolar septa thickened due to infiltrating cells.

Preliminary exposures using intermittent exercise at twice resting metabolic rate indicated that the exercise greatly enhanced the abundance of focal parenchymal lesions and that

lesion response was greater than that predicted by a simple proportion to ventilation dose rate (Mautz et al., 1982). An exposure was then performed to examine the effects of increasing exercise workload while effective dose of O_3 was held constant by decreasing exposure duration to compensate for increased metabolic rate. Duration was thus adjusted to hold the quantity (ppm O_3) · (duration) · (average $\dot{V}_{O2}$) constant. Exercise levels and corresponding exposure durations were continuous rest (3.4 h), running 8 m/min at 0% grade (2.75 h), 15 m/min at 20% grade (2.33 h), and 30 m/min at 20% grade with 2 rest periods of 7 min (1.75 h). Despite equivalence of effective dose of O_3, lesion areas increased with increasing exercise intensity (Figure 1). Type I lesion areas for rest and 8 m/min, 0% grade groups were similiar to control, but increased by a factor of 1.5 and 3 for exercise at 15 m/min, 20% grade and 30 m/min, 20% grade, respectively. A low incidence of Type II lesions was observed in resting rats; however, the area incidence increased greatly with increasing exercising levels at shorter exposure durations. These results demonstrate the critical importance of exercise as a modifying factor and the inability of the effective dose concept to explain variation in toxic responses to ozone inhalation during exercise.

Exercising Dogs Exposed to 10 ppm Formaldehyde

The effects of formaldehyde on pulmonary function during exercise were studied in purebred female beagle dogs. Dogs were housed in pure air kennels for at least six weeks prior to exposure, and they were trained to run on a refrigerated treadmill while wearing a light weight, low dead space respiratory mask (Stavert et al., 1982 A,B). The treadmill refrigeration system was computer controlled to regulate dog body temperature below the threshold for panting respiration (Reischl et al., 1982). Pulmonary function parameters were measured while the dogs were exposed and exercising on the treadmill. Respiratory gases were analyzed at the mouth by a mass spectrometer. Inspiratory flow, expiratory flow, % CO_2 and % O_2 esophogeal pressure, rectal temperature, and skin temperature were simultaneously recorded on a strip chart recorder, an FM instrumentation tape recorder, and a computer. Breath-by-breath analysis of pulmonary function signals yielded measurement of $\dot{V}_{O2}$, $\dot{V}_{CO2}$, breath time, inspiratory and expiratory times, inspired and expired tidal volumes, maximum inspiratory and expiratory flow rates, minute ventilation, and a dynamic measure of pulmonary resistance and compliance (Reischl et al., 1981; Beaucage and Reischl, 1981). A single exercise exposure test was 200 minutes, and breath-by-breath pulmonary function data were collected for 2-4 min at 20 min intervals for a total of 10 data points.

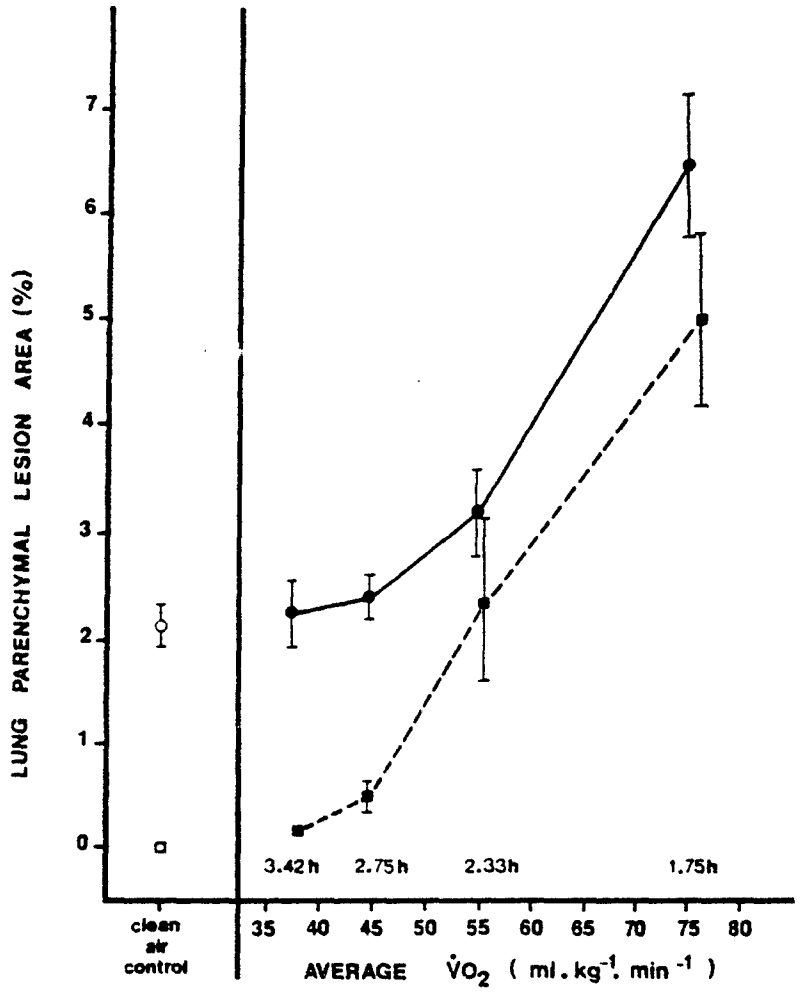

Figure 1. Effect of exercise on lung parenchymal lesions induced by 0.35 ppm O_3 at constant effective dose. Data are plotted as a function of average $\dot{V}_{O2}$ for different exercise intensities. Exposure durations are listed along the abscissa. Data are mean ± SE of Type I (circles) and Type II (squares) lesion areas. Sample sizes were 8-10 for each group.

The first data point was recorded after the dog stood at rest, the second after a warmup exercise period of 5 km/h running at 0% grade. For data points 3-9, the dogs exercised continuously at 5 km/h and 7.5% grade which elevated ventilation and $\dot{V}_{O2}$ above resting rates by a factor of 2. The total exercise period at grade was 120 min and formaldehyde exposure followed collection

of the third data point. The final data point was recorded with the dogs standing at rest and continuing to breathe formaldehyde. Data point 3 provided an initial set of measurements during exercise at grade in clean air for comparison to subsequent exercise in the formaldehyde atmosphere (data points 4-9). Each dog ran through the entire protocol in clean air 2 days before a formaldehyde exposure run. A third postexposure run was conducted in clean air on the day following formaldehyde exposure. 5 dogs were used and each animal was tested 2-3 times.

Exercise affected pulmonary function by reducing total dynamic pulmonary resistance and compliance during 120 min of running. Respiratory rate, tidal volume, $\dot{V}_{O2}$, and $\dot{V}_{CO2}$ increased. It was expected that the effects of formaldehyde on pulmonary function would be superimposed on the effects of exercise; the demands of exercise on ventilation and gas exchange could override the effects of inhaled formaldehyde or, on the other hand, increased dose rate and redistribution of the inhaled pollutant could potentiate pulmonary function effects.

Exercise resulted in a decline in pulmonary resistance to 40% of initial resting value (Figure 2). During formaldehyde inhalation, resistance did not decline to the same degree but was elevated above control clean air levels. Breath time (Figure 3) and expired tidal volume were increased during the first two thirds of formaldehyde exposure, a breathing pattern similar to reflex slow deep breathing observed in resting rodents inhaling sensory irritants (Chang et al., 1981). The reflex pattern was not suppressed by exercise, but it was not persistent throughout the exposure. Breath time and expired tidal volume returned to clean air values during the last third of the exposure; however, the recovery did not result in a completely normal exercise breathing pattern. Fractional expiration time (time expiration/total breath time) was increased in the latter phase of formaldehyde exposure, a feature consistent with the observed elevation of pulmonary resistance throughout the exercise exposure.

The above examples of pulmonary function changes illustrate the degree to which small effects can be detected in exercising exposures. Data from subjects at rest exhibit much larger variation (Figures 2 and 3) probably due to the potential for differences in posture, distraction, and excitement to influence respiration. Exercise involves more of the functional reserve of the respiratory system and reduces variability in measured pulmonary function parameters.

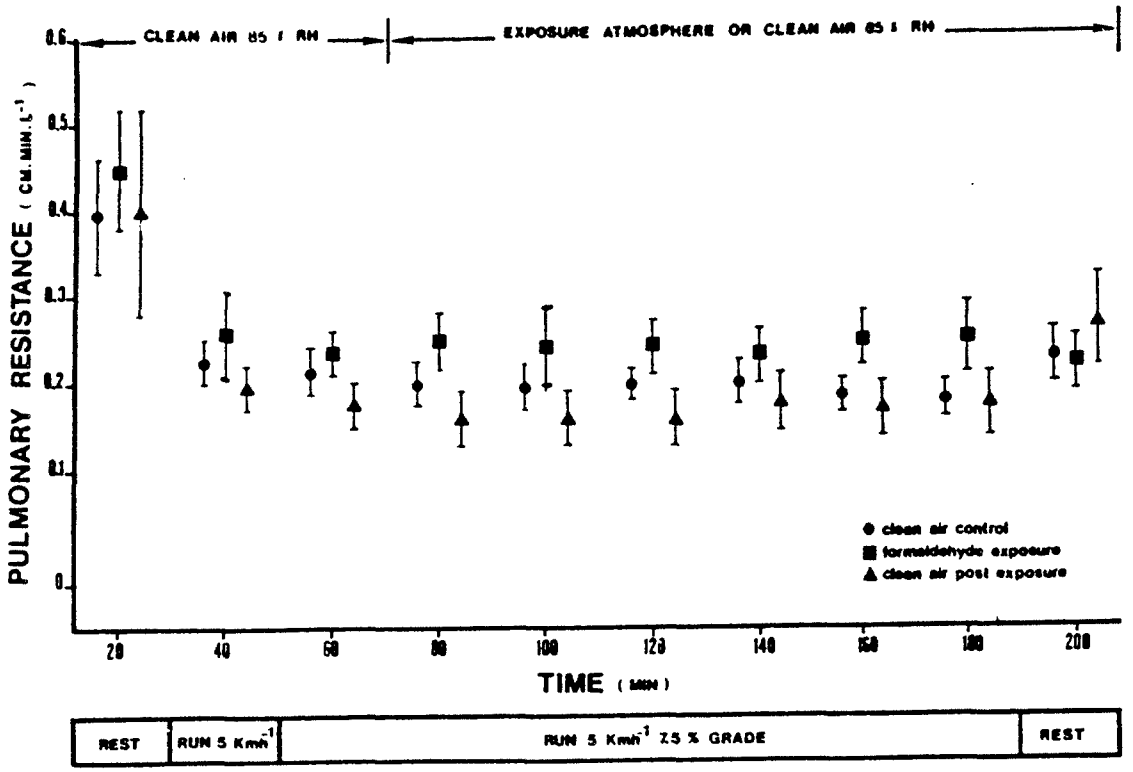

Figure 2. Pulmonary resistance (mean ± SE, n=10) of beagle dogs at rest and exercise. Clean air control run (circles) preceeded 10 ppm formaldehyde run (squares) by 2 days; post-exposure run (triangles) was one day later.

Reflex changes in breathing pattern in response to respiratory irritants were not abolished by exercise ventilation, and reduced variation in breathing pattern parameters can make these effects easier to detect. Studies of oxidant induced damage in exercising rodents have shown how relatively small increases in dose rate produced by exercise ventilation lead to large increases in tissue injury, and in many cases concentrations of inhaled pollutants which show no measurable effects in resting exposures can result in significant adverse effects when inhaled during exercise. Exercising animals should continue to offer a realistic and sensitive model for toxicology testing.

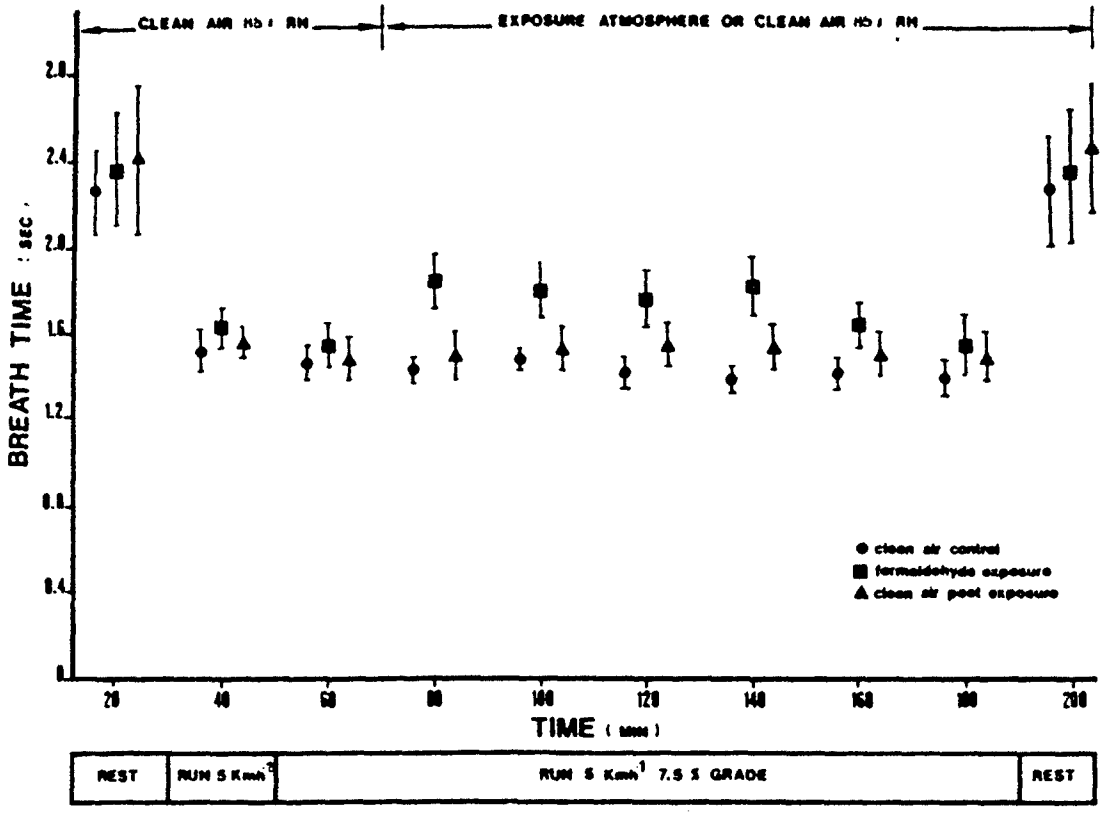

Figure 3. Breath time (mean ± SE, n=12) of beagle dogs at rest and exercise. Clean air control run (circles) preceeded 10 ppm formaldehyde run (squares) by 2 days; post-exposure run (triangles) was one day later.

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