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# A rodent treadmill for inhalation toxicological studies and respirometry

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MAUTZ, WILLIAM J., ROBERT F. PHALEN, THOMAS R. McCLURE, AND CHARLES BUFALINO. *A rodent treadmill for inhalation toxicological studies and respirometry.* J. Appl. Physiol. 58(2): 673–679, 1985.—A 10-runway treadmill was enclosed for inhalation toxicological studies of rodents under exercise exposure to environmental pollutants. The exposure system was lined with sheet stainless steel to minimize scrubbing of charged particles and reactive gases. Average metabolic gas exchange of exercising animals was derived from measurements of inlet or outlet airflow and data from an $O_2$ analyzer in conjunction with either a $CO_2$ or $N_2$ analyzer. An airflow rate of $400 \, l \cdot min^{-1}$ ensured a response time of 1 min to reach 95% of a step change in metabolic rate and held scrubbing losses of an $O_3$ test atmosphere to less than 2% of treadmill inlet concentration. Gas exchange averaged for 10 rats during incremental exercise up to their highest collective performance was similar to published data for rats tested individually.

inhalation exposure apparatus; air pollutant; metabolic gas exchange; rodent exercise

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ALTHOUGH INHALATION EXPOSURES of laboratory animals are commonly performed at rest, there are good reasons to conduct studies during exercise. In many occupational and environmental human exposures, people working or playing have significantly elevated ventilation rates. In contrast, it is not uncommon to observe experimental animals maintaining minimal states of activity, in many cases apparently sleeping, during exposures in inhalation chambers. In studies using animals as models of human inhalation exposure the lack of similarity in such cases is striking.

Several events occur during exercise that are known or suspected to alter the biological effects of a given concentration of an airborne toxin. Increased minute ventilation will lead to greater total internal exposure doses per unit time. For the average adult human, resting minute ventilation is about $7 \, l \cdot min^{-1}$. During brief heavy exercise an increase to over $100 \, l \cdot min^{-1}$ has been observed (3). Thus exercise can increase the rate of delivery of an inhaled toxin by more than 10-fold. Other events occurring during exercise that may modify response in-clude greater depth of breathing, decreased contact times between inspired air and upper airways, an increase in the fraction of air drawn through the mouth (rather than the nose), and the potential overriding of reflex-mediated protective breathing patterns (e.g., shallow breathing) that are normally stimulated by irritants (2, 6, 28). In general, higher flow rates will change the deposition efficiencies for gases and particles in all regions of the respiratory tract, leading to a redistribution of the dose pattern and possibly a change in the total combined dose delivered to all regions. In many cases these factors would be expected to lead to increased responses to inhaled pollutants. Indeed, inhalation studies with both humans and laboratory animals have typically demonstrated greater responses during exposures at exercise than were seen at rest (9, 12, 22–24).

For the purpose of examining the effect of exercise as a modifier of the toxic response to air pollutant compounds, an enclosed treadmill exposure system was developed for use with rats as an exercising animal model. The treadmill was designed to simultaneously expose 10 running animals to test gases or aerosols and to permit simultaneous measurement of group respiratory gas exchange as an index of metabolic work load during exposure. The treadmill was used to determine the relationship between running speed and metabolic gas exchange at two elevation grades (0 and 20%). In addition, procedures were tested for measuring $O_3$ concentration in the treadmill with rats present.

## METHODS

*Treadmill design.* The treadmill exposure system was constructed from a Quinton 42-15 rodent treadmill (Fig. 1). The treadmill contained 10 individual runways and controls for varying belt speed and inclination. An electrically charged grid at the rear of the belts motivated the animals to continue exercise. The treadmill was enclosed with polycarbonate plastic (6 mm) and sealed against significant leaks with neoprene gaskets. The drive motor was external to the enclosure, and a seal on the belt drive shaft was effected with a pair of semicir-
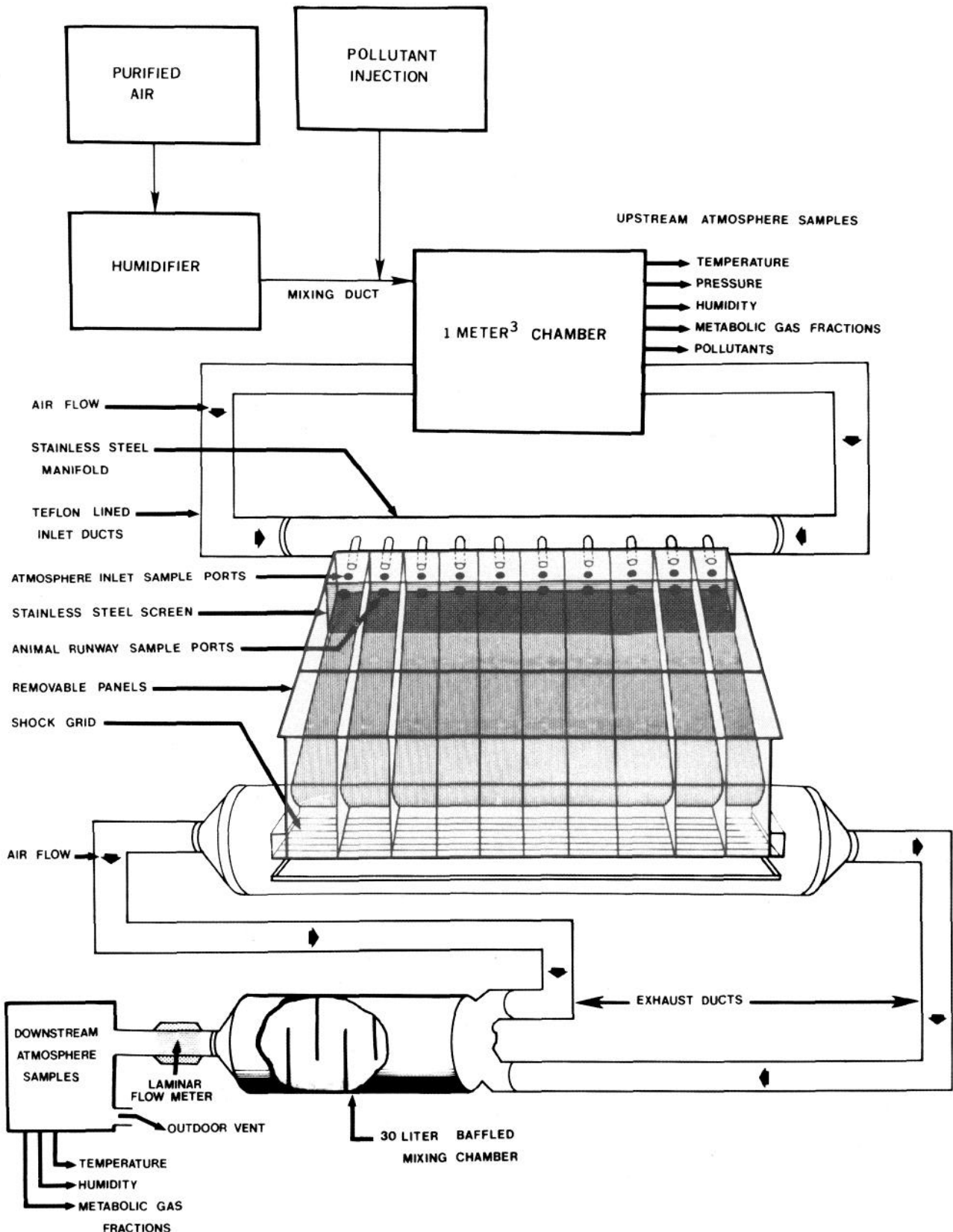
FIG. 1. Rodent treadmill exposure system. Test atmosphere is individually delivered to 10 animals running in separate channels. Exposure atmosphere can be sampled at several positions and metabolic rate is measured as an average for the animal group. See text for details.

cular Teflon bushings mounted in an aluminum panel. Inner surfaces of the runways were lined with a stainless steel sheet to minimize both electrostatic charge effects and reaction with corrosive atmospheres. Inlet ports were centered at the front of each runway. One-centimeter-diameter stainless steel disk baffles mounted 1 cm behind the ports and stainless steel screens (2 × 2 mesh) positioned at 10 cm distributed the atmosphere uniformly in

the runways and prevented rats from contacting inlet ports.

Removable panels above the charged grid provided access to individual runways. Another removable panel spanning the front of the treadmill below the inlet ports gave access to belt tension adjustment screws and to the enclosure floor beneath the belts. Excreta fell onto a removable tray on the enclosure floor and fecal masses clinging to the belts were brushed loose with wire wipers mounted to press lightly on the belts. During operation the belts became wet with urine and spotted with smeared fecal matter. This raised concern about the possible development of significant ammonia levels in the treadmill although continuous inflow of exposure atmosphere made this unlikely. $NH_3$ concentration was measured from the treadmill while rats performed intermittent exercise and rest. Atmosphere was sampled from a runway, and $NH_3$ was collected into 0.05 M $H_2SO_4$. The solution was adjusted to pH 13, and $NH_3$ concentration was measured with an ion-selective electrode (HNU Systems, Newton, MA). Airborne $NH_3$ concentration was found to be less than 0.1 ppm.

A pair of exhaust lines at the rear of the treadmill conducted the atmosphere to a 30-liter baffled mixing chamber, then through a flowmeter to sample ports for humidity and metabolic gas fraction measurement and to an outdoor vent blower. Air inflow and outflow rates were adjusted to regulate the treadmill at ambient pressure.

Exposure atmospheres were produced by mixing test compounds or aerosols with purified air and delivering the mixture to each runway of the treadmill. Supply air was initially compressed and purified by passage through Purafil (Atlanta, GA) and Del-Monix (Deltech, Newcastle, DE) gas scrubbing filters, then decompressed, passed through a high-efficiency particulate absolute (HEPA) filter, and humidified by controlled injection of water vapor. The mixed atmosphere was passed to a 1-m$^3$ stainless steel chamber containing a temperature and humidity sensor (Hygrodynamics, Silver Spring, MD), then through paired Teflon-lined flexible ducts to a stainless steel manifold that distributed the flow to the 10 treadmill runways. A pair of atmosphere sample ports was located in each runway at positions 3 cm upstream and 3 cm downstream of the stainless steel screen barriers. The first port withdrew samples of the inlet atmosphere and the second withdrew the atmosphere in the immediate vicinity of the rats.

*Metabolic rate measurement.* Average metabolic rate of 10 rats exercising in the treadmill was determined by analysis of input and output fractional gas composition, water vapor content, and flow rate (see APPENDIX for details.) Measurements were made by alternately sampling upstream and downstream air with a mass spectrometer (Perkin-Elmer model 1100, Pomona, CA) calibrated with gravimetric standard gases (Liquid Carbonics, Los Angeles, CA). Atmosphere flow rates were measured either with a single Fleisch no. 4 pneumotachograph (Dynascience, Blue Bell, PA) in the exhaust stream or paired Fleisch no. 1 pneumotachographs on the input manifold in conjunction with differential pressure transducers (Validyne MP-45 Northridge, CA). Hu-

midity and temperature were measured with a dew point sensor (EG&G model 911, Waltham, MA). Output of all instruments was displayed on a chart recorder (Gould model 2800, Cleveland, OH) and digitized at 40 Hz for calculation of respiratory gas exchange with a PDP 11-10 computer system (Digital Equipment, Maynard, MA). To perform a measurement under steady-state conditions, downstream and upstream gas fractions, along with atmosphere flow rate, temperature, and humidity, were sampled five times over a 30-s period. Gas exchange was then calculated by computer from mean values of the variables and expressed as the mean value per individual rat based on the number of animals in the treadmill.

Gas turnover kinetics of the treadmill atmosphere were evaluated by analyzing the response to step changes in a calibration gas input to treadmill runways. An exponential first-order relation between outflow gas fraction and time provided an adequate fit to the treadmill response. Logarithmic transformation of gas fraction data yielded a straight-line relation to time with slope $1/t$ and intercept $-d/t$, where $t$ is the time constant and $d$ is the delay time (19).

Correlation coefficients for repeated trials ($n$ = 8) ranged from 0.97 to 0.99 for atmosphere flow rates of 400 or 100 l·min$^{-1}$. Time constants at these flow rates were 10 and 51 s, respectively, and corresponding delay times were 29 and 81 s. These values resulted in an expected time to reach 95% of steady-state gas fraction following a step change of 59 s at 400 l·min$^{-1}$ input flow and 233 s at 100 l·min$^{-1}$ input flow.

For gas exchange measurements, the system was tested before and after an experiment with gravimetric standard calibration gas elevated in $CO_2$ and depressed in $O_2$ composition. Calibration gas was bled into the treadmill runways, and expected values of $O_2$ consumption ($\dot{V}O_2$) and $CO_2$ production ($\dot{V}CO_2$) were calculated from *Eqs. 5* and *6* or *7* and *8* (APPENDIX) using calibration gas composition as output fractions, treadmill inlet gas composition as input fractions, and metered flow rate (Bubble Meter, SKC West, Fullerton, CA) of calibration gas into the treadmill.

*Animals, training, and exercise protocol.* The relation between metabolic rate and running speed was determined for Sprague-Dawley rats (Hilltop Lab Animals, Chatsworth, CA) with mean weight at testing of 272 ± 21 g. The animals were trained to run at level grade in the treadmill over a 3-day period commencing with 15 min of running at 8 m·min$^{-1}$ alternating with 5 min of rest for 1.6 h. On the second day rats alternated 30 min of running with 10 min of rest for 3.2 h, and on the third day they ran continuously for 3 h. The purpose of the training protocol was to acquaint the rats with the treadmill and shock grid and to identify individuals that would not run readily in the apparatus. About 3% of the animals could not be trained to run reliably and were excluded from the experiment. Metabolic rate was measured from groups of 10 rats at an airflow through the treadmill of 400 l·min$^{-1}$. Following initial measurements at rest, treadmill speed was set at 10 m·min$^{-1}$ at level grade, and speed was increased in step changes of 5 m·min$^{-1}$ at 6-min intervals. Metabolic rate was measured at each step

after output flow gas fractions stabilized, and the test was terminated when rats failed to maintain continuous exercise. Additional sets of measurements were made at 20% grade with 10 m·min⁻¹ step increases over the lower range of speeds.

*Pollutant atmosphere.* An ozone ($O_3$) atmosphere was used to test treadmill distribution of a pollutant compound and assess possible scrubbing by reactive components of the treadmill inhalation system. $O_3$ was produced by passing medical-grade $O_2$ through an electrostatic discharge $O_3$ generator (Sander Ozonizer, type III, Osterberg, FRG) and diluted into the humidified airstream. Samples of the exposure atmosphere were drawn in succession from three positions in the atmosphere stream: *1*) in a 1-m³ stainless steel chamber just before the atmosphere entered the treadmill manifold, *2*) in the treadmill runways between the inlet ports and stainless steel screens, and *3*) in the treadmill runways behind the stainless steel screen. Samples were drawn through Teflon tubing, and $O_3$ concentration was measured by ultraviolet spectrophotometry in a Dasibi model 1003-AH $O_3$ analyzer. The third position most closely approximated the rat breathing zone. However, because the rats had direct access to these sample tubes, samples could contain expired respiratory air or air aspirated through fur. $O_3$ distribution in the treadmill was measured with no animals present, animals present at rest, and animals exercising at 10.7 m·min⁻¹.

## RESULTS

The relation between running speed and metabolic gas exchange for groups of 10 rats running at level grade and 20% grade is shown in Fig. 2. On level grade, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ increased linearly between 10 and 20 m·min⁻¹. At higher speeds the response was attenuated as the rats approached exhaustion, and the experiment was terminated at 40 m·min⁻¹ as animals became unable to continue running. Rats running at 20% grade exhibited only slightly higher rates of metabolic gas exchange at speeds < 30 m·min⁻¹; however, the response was linear over the entire range of speeds at which they were able to perform.

Distributions of $O_3$ concentrations in parts per million (ppm) in the treadmill exposure system are shown in Table 1. $O_3$ losses between the 1-m³ chamber and the first sample ports upstream of the stainless steel screens were 5–6%. When rats were not present in the treadmill, and additional 1–2% loss occurred in passage to the second sample port behind the screens. When rats were present in the treadmill losses measured in the runways were greater. With animals running, the measured loss between sample ports was 5%, and when the animals were at rest in the treadmill the loss was 20%. However, mass spectrometer analysis of air samples from the second sample ports behind the screens revealed elevated and highly variable $CO_2$ fractions which indicated that these samples contained expired respiratory air. Variation in $O_3$ concentration among runways was statistically significant ($F = 7.3$, $P < 0.001$) for samples upstream of screens when rats were present, however the runway differences were not large. Mean concentrations in individual runways measured at upstream sample ports with rats present ranged from 0.372 to 0.382 ppm with
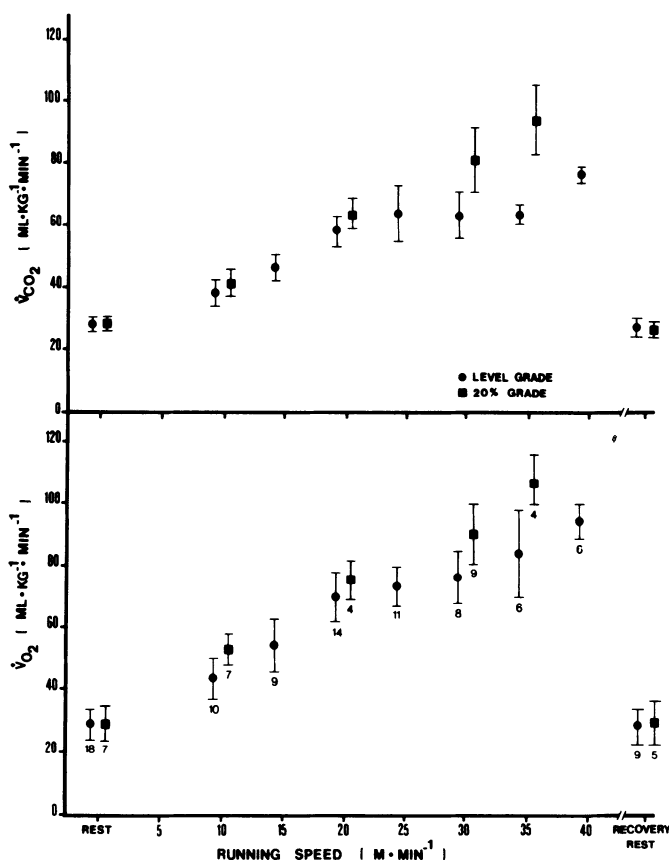


FIG. 2. Metabolic gas exchange (STPD) of groups of 10 rats as a function of running speed on level grade (*circles*) or 20% grade (*squares*). Data are means ± SD, and numbers are sample sizes of measurements from animal groups. Means have been offset to avoid overlap; measurements were made at the speeds listed.

TABLE 1. *$O_3$ concentrations (ppm) achieved in the treadmill exposure system*

| | Treadmill Running, 11 m·min⁻¹ | | | Treadmill Off | | |
|---|---|---|---|---|---|---|
| | Mean ± SD | n | Range | Mean ± SD | n | Range |
| | *No Rats Present* | | | | | |
| 1-m³ source chamber | 0.359 ±0.011 | 20 | 0.335 –0.375 | 0.355 ±0.014 | 20 | 0.330 –0.375 |
| Upstream of runway screens | 0.341 ±0.009 | 20 | 0.326 –0.351 | 0.333 ±0.010 | 20 | 0.315 –0.348 |
| Downstream of runway screens | 0.336 ±0.010 | 20 | 0.314 –0.348 | 0.326 ±0.012 | 20 | 0.311 –0.347 |
| | *Rats Present* | | | | | |
| 1-m³ source chamber | 0.401 ±0.009 | 5 | 0.391 –0.413 | 0.398 ±0.006 | 7 | 0.389 –0.406 |
| Upstream of runway screens | 0.377 ±0.005 | 86 | 0.361 –0.387 | 0.375 ±0.006 | 183 | 0.357 –0.389 |
| Downstream of runway screens | 0.359 ±0.014 | 42 | 0.316 –0.382 | 0.300 ±0.050 | 78 | 0.184 –0.384 |

Data are combined for all 10 runways, and when rats were present, sampling emphasized exposure region. Input flow rate was 400 l·min⁻¹.

the treadmill running and from 0.371 to 0.379 ppm with the treadmill off.

## DISCUSSION

Exercise is expected to be an important modifier of the toxic effects of inhaled pollutant compounds and a

number of investigations have shown that exercise exposure to pollutant compounds exacerbates induced changes in pulmonary function of humans (1, 9, 22). With the development of exercising animal models, inhalation toxicological studies may address questions that cannot be approached with human subjects, such as exercise effects on histopathology and biochemistry. The enclosed multichannel treadmill system described here provided an efficient means for exposing up to 10 exercising rats simultaneously and uniformly to a controlled atmosphere. A preliminary experiment with the apparatus (16) demonstrated exercise enhancement of ozone-induced lung lesions.

Fulfilling a basic requirement to contain exposure atmosphere in the treadmill serves in adapting the system for open flow respirometry and quantifying metabolic work load of the exercising animals. The choice of computational method for measuring metabolic gas exchange is governed by available instrumentation and practical considerations of exposure system design. The method presented (APPENDIX) is based on water vapor free gas fractions and correction of atmosphere flow to STPD. Techniques with single gas analyzers with and without water vapor and $CO_2$ absorption have been described (10, 11, 14, 27), and other sampling arrangements and equations appropriately derived from mixed and component gas flows (*Eqs. 1–4,* APPENDIX) may be used. High flow rates in inhalation exposure systems make gas scrubbing of the total flow impractical; however, samples may be subjected to differential absorption before analysis. One may choose to measure input flow or output flow and apply different gas exchange equations for the two cases. If there are any leaks in the treadmill enclosure, an output flow measurement is preferable. The treadmill may then be operated under slightly negative pressure so that any leakage of air is directed inward, mixed with respired gas, and included in the measurements of total flow and output metabolic gas fractions. There will be no error in metabolic rate estimates unless respiratory gas fractions of the inlet exposure atmosphere differ from room air. If input flow is measured, any leakage (outward before complete mixing or inward) will alter output metabolic gas fractions and not be accounted in the flow measurement. Airflow rate through the treadmill must be set sufficiently high to ensure uniform exposure atmosphere distribution and an adequate response time to changes in metabolic rate; however, the flow must not be so great as to compromise resolution of metabolic gas fractions. We found a flow of 400 l·min$^{-1}$ appropriate for exposing 10 rats and measuring gas exchange in the treadmill system.

The relation between running speed and metabolic gas exchange for rats running in the exposure system was in agreement with data reported in other studies of exercise metabolism of laboratory rats (4, 5, 7, 13, 18, 20, 21). There is a large degree of variation in results of these separate studies, and most investigators believe that regression slopes, presence of metabolic plateaus, and the values of maximum $O_2$ consumption ($\dot{V}O_{2 max}$) and its corresponding running speed depend on animal training, exercise protocols, and selection criteria for performance (4, 13). Data for exercising Sprague-Dawley rats of mass similar to our animals (4, 5, 13, 21) fall within the range of results reported here (Fig. 2). Gleeson and Baldwin (13) reported a sharp plateau in $\dot{V}O_2$ of untrained rats performing incremental exercise, and others (5, 7, 18, 20, 21) have observed attenuation of metabolic response or plateaus with increasing running speed. In our experiments $\dot{V}O_2$ continued to increase with increments of running speed, but while the response remained linear for running at 20% grade, an attenuated response and lower peak $\dot{V}O_2$ were observed for level running. It is possible that attenuation would have been observed at 20% grade if additional measurements were made between 30 and 35 m·min$^{-1}$. However, there was an important difference between level and 20% grade protocols; rats running uphill had larger speed increments and shorter total exercise duration to reach peak sustained speed. Thus rats running at 20% grade became exhausted after four speed steps (18–24 min of running), whereas rats on level grade attained seven speed steps (36–42 min of running). Extended incremental exercise protocols may induce exhaustion and refusal to run at submaximal exercise levels. In another study using rats trained for several weeks, selected for superior running performance, and tested directly at each speed without progressive incremental increase (4), metabolic plateaus were not observed and the animals were capable of performing at speeds up to 50 m·min$^{-1}$ at 17.8% grade. The diversity of metabolic responses and exhaustion behavior of rats under conditions of maximal exercise has made it difficult to precisely and reproducibly characterize $\dot{V}O_{2 max}$. The highest $\dot{V}O_2$ measured in the present study (103 ± 14 ml·kg$^{-1}$·min$^{-1}$) fell within the range of $\dot{V}O_{2 max}$ values reported in other studies using both incremental and single speed protocols (5, 7, 13, 20, 21), although our values were based on repeated measurements of the collective sustained performance of 10 animals and might be expected to underestimate an average of individual animal measurements. In view of the variability of $\dot{V}O_{2 max}$ determinations and frequent absence of definitive physiological indicators such as metabolic plateaus and continuous lactate accumulation (4, 5, 25), any investigation of the effects of toxic substances on maximal metabolic rate must use control groups strictly matched in age, size, and exercise experience and must consider the influence of running behavior on the end point.

Characterization of inhaled pollutant concentration is an important problem in inhalation toxicology, as particles and gases may be scrubbed from the test atmosphere along the delivery path. In addition, atmosphere sampling in the breathing zone of test animals introduces the possibility of contaminating the sample with expired respiratory gas. Ideally one would like samples of inhaled concentration at the mouth and nose such as might be obtained with a respiratory mask and valve. For exposures of animals in chambers, however, inhaled concentration is usually assumed to be equivalent to that of inlet air. With sufficient input flow, losses to the chamber structure can be minimized, but evasive maneuvers by test subjects such as tucking the head in the fur remain a potential problem. Rats exercising in the enclosed treadmill system were advantageously exposed head-on to a continuous flow of test atmosphere. Samples drawn

directly from the runway in the breathing zone of the exercising animals were often spoiled by collection of expired respiratory gas, and this problem was greatest when the animals were at rest. Resting animals spent considerable time in close proximity to the sample line. The most representative atmosphere samples were obtained upstream, separated from the animals by coarse mesh stainless steel screens. Exhalant gas was not present in these atmosphere samples and with no animals present in the treadmill, losses of $O_3$ flowing down the runway were minimal (Table 1).

## APPENDIX

Methods for measuring metabolic gas exchange in open flow respirometers have been developed for several particular cases (7, 8, 10, 11, 14, 15, 26, 27). All these methods can be related by a set of equations describing the balance flow of each component gas in question and the total flow rate of mixed atmosphere into and out of the respirometer. By substitution, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ may be expressed in a minimum number of variables appropriate to available instruments. Symbol terminology follows earlier analyses (10, 11, 14, 27).

| | |
|---|---|
| $\dot{V}_{O_2}$ | rate of $O_2$ consumption of animals in the treadmill (STPD) |
| $\dot{V}_{CO_2}$ | rate of $CO_2$ production of animals in the treadmill (STPD) |
| $\dot{V}_{N_2}$ | rate of $N_2$ exchange (STPD) assumed to be zero |
| $\dot{V}_I$ | rate of airflow into the treadmill (STPD) |
| $\dot{V}_E$ | rate of airflow out of the treadmill (STPD) |
| $F_{I_{O_2}}$ | dry volume fraction of $O_2$ in inlet air |
| $F_{E_{O_2}}$ | dry volume fraction of $O_2$ in output air |
| $F_{I_{CO_2}}$ | dry volume fraction of $CO_2$ in input air |
| $F_{E_{CO_2}}$ | dry volume fraction of $CO_2$ in output air |
| $F_{I_{N_2}}$ | dry volume fraction of $N_2$ in input air |
| $F_{E_{N_2}}$ | dry volume fraction of $N_2$ in output air |

The basic equations for volume flow rates of component gases are

$$\dot{V}_{O_2} = \dot{V}_I F_{I_{O_2}} - \dot{V}_E F_{E_{O_2}} \tag{1}$$

$$\dot{V}_{CO_2} = \dot{V}_E F_{E_{CO_2}} - \dot{V}_I F_{I_{CO_2}} \tag{2}$$

$$\dot{V}_{N_2} = \dot{V}_E F_{E_{N_2}} - \dot{V}_I F_{I_{N_2}} = 0 \tag{3}$$

The relation between total input and output volume flow rates is

$$\dot{V}_E = \dot{V}_I - \dot{V}_{O_2} + \dot{V}_{CO_2} \tag{4}$$

*Equations 1* and *2* contain both input and output airflow terms, $\dot{V}_I$ and $\dot{V}_E$. By rearranging *Eq. 4* and substituting for either $\dot{V}_I$ or $\dot{V}_E$ in *Eqs. 1* and *2*, respiratory gas exchange may be expressed in terms of either the input or the output flow, whichever is more conveniently measured. Both terms $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are introduced into each of the equations by this substitution, but by simultaneous solution of the two equations for these two variables, expressions for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are obtained in terms of input and output fractional gas concentrations and a total airflow rate, be it either input or output flow. For the case in which $\dot{V}_E$ is measured, these expressions are

$$\dot{V}_{O_2} = \dot{V}_E \frac{F_{I_{O_2}}(1 - F_{E_{CO_2}}) - F_{E_{O_2}}(1 - F_{I_{CO_2}})}{1 - F_{I_{O_2}} - F_{I_{CO_2}}} \tag{5}$$

$$\dot{V}_{CO_2} = \dot{V}_E \frac{F_{E_{CO_2}}(1 - F_{I_{O_2}}) - F_{I_{CO_2}}(1 - F_{E_{O_2}})}{1 - F_{I_{O_2}} - F_{I_{CO_2}}} \tag{6}$$

Alternatively, $\dot{V}_I$ or $\dot{V}_E$ may be eliminated from *Eq. 1* and *2* by rearrangement and substitution of *Eq. 3*. This procedure yields expressions with the Haldane transformation using $F_{I_{N_2}}$ and $F_{E_{N_2}}$ (8). Again, the equations may be derived for measurements of either $\dot{V}_I$ or $\dot{V}_E$. The case for $\dot{V}_E$ measurement is

$$\dot{V}_{O_2} = \dot{V}_E \left[ F_{I_{O_2}} \frac{F_{E_{N_2}}}{F_{I_{N_2}}} - F_{E_{O_2}} \right] \tag{7}$$

$$\dot{V}_{CO_2} = \dot{V}_E \left[ F_{E_{CO_2}} - \frac{F_{E_{N_2}}}{F_{I_{N_2}}} F_{I_{CO_2}} \right] \tag{8}$$

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