Quantitation of Pulmonary Fluid Changes Due to Whole-Body Vibration in the Mouse* (33918)

ALBERT I. KING, ROY ASTON, AND DONALD J. VANKIRK

Department of Mechanical Engineering Sciences, College of Engineering, and Department of Anesthesiology, School of Medicine, Wayne State University, Detroit, Michigan 48202

Animals succumbing to low frequency vibration exhibit gross pulmonary and gastrointestinal tissue damage. The severity of lung injury, as judged by the degree of alveolar hemorrhage produced, has been reported to correlate with the intensity of vibration exposure (1). Schaefer *et al.* (2) observed pulmonary hemorrhage, atelectasis, emphysema, and edema in rats dying under exposure to whole-body vibration. Similar results in the mouse were reported by Aston and Roberts (3) who found, in addition, that tolerance to vibration could be enhanced by central depressant agents. The protection afforded by these drugs correlated with a reduction in the incidence and severity of pulmonary damage observed at autopsy.

Since both pulmonary hemorrhage and edema have been reported in vibrated animals, it seems pertinent to know which response correlates with lethality in order to investigate the mechanism of pharmacological protection against vibration-induced death. In the present study, therefore, both pulmonary blood content and pulmonary edema were quantified in vibrated and control mice.

Materials and Methods. Parameters of Vibration. A vibration tester (MB, model C 31), producing simple harmonic motion over a wide range of frequencies, was calibrated to provide 0.313-in. displacement at a frequency of 25 Hz. These parameters produced a peak acceleration of $\pm 10g$ and were chosen to replicate conditions previously employed in this laboratory (3). Experimental animals were held in centrifuge tubes inserted into a wooden rack, on the top of which was fixed an accelerometer (Endevco, model 2215) coupled to an oscilloscope. Five mice were vibrated at one time. Each animal produced an intermittent single spike on the oscilloscopic tracing while alive, which became persistent as the mouse approached the point of expiration.

A strobe light was used to permit continuous visual examination of the animal to detect body motion and the withdrawal reflex elicited when a heat source was placed in proximity to the snout. Persistency of the spike on the oscilloscope trace, and the absence of body motion and reflexes all served to indicate imminent death. The animal was removed from the test rack as soon as these signs appeared.

A total of 55 male mice of the Swiss Webster ICR strain weighing between 24 and 28 g were used. Animals were held in isolation in animal quarters for 4 days, to allow acclimation to conditions of housing and diet. On the fifth day the mice were separated in groups of 5 in cylindrical metabolic cages, for a minimum of 30 min, before testing.

Forty-one mice were subjected to whole-body vibration for a maximum of 30 min. A total of 26 mice expired within this period while the surviving 15 animals were sacrificed to determine if any significant pathologic changes had occurred. Pulmonary blood content and the degree of pulmonary edema were calculated in all these mice as well as in a nonvibrated control group of 14 animals which were sacrificed using sodium thiopental.

Analysis of lung fluid changes. Pulmonary blood content was calculated by extracting hemoglobin from coarsely chopped lung tissue into 0.48% aqueous ammonia solution (30 ml/g of lung tissue) by mechanical agitation for 1 hr, following a modification of the hemoglobinometric method described by

* A preliminary report of this study was presented at the Twenty-first Annual Conference on Engineering in Medicine and Biology, Nov. 17–21, 1968 in Houston, Texas. This study was supported, in part, by USPHS Research Grant UI-00056-10.

TABLE I. Mean Weights ± SE for Whole Lung and Pulmonary Blood and Edema Fluid (g/kg of body wt) in Control and Vibrated Mice.

Group	No. of mice	Lung wt	Lung blood	Edema fluid
Control	14	5.86 ± 0.15	0.947 ± 0.035	—
Vibrated (survived)	15	6.47 ± 0.25[a]	0.957 ± 0.043	0.597 ± 0.254
Vibrated (expired)	26	7.18 ± 0.20[a]	1.399 ± 0.079[a]	0.912 ± 0.172

[a] Significantly different from control value ($p = 0.05$).

Wintrobe (5). The ammonia extract was cleared by filtration and the lung tissue was extracted a second time. The two extracts were combined and the hemoglobin content was measured colorimetrically at a wavelength of 540 mμ. Total lung hemoglobin was converted to grams of whole blood, assuming an average mouse hemoglobin concentration of 15 g/100 ml of blood (6).

Pulmonary edema was quantified on the basis of wet weight of whole lung expressed as a percentage of preoperative body weight, as suggested by Visscher *et al.* (4). However, any increase in lung weight resulting from vibration was assumed to reflect both edema fluid and an increase in blood content. The amount of pulmonary edema for each vibrated mouse, therefore, was quantified by letting

$$\Delta W_v = L_v - \overline{L}_c - (B_v - \overline{B}_c),$$

where ΔW_v is the increase in lung water, i.e., edema, due to vibration, L_v is the lung weight after vibration, $\overline{L}_c$ is the mean control lung weight, B_v is the calculated lung blood weight after vibration, and $\overline{B}_c$ is the mean control lung blood weight. All values are expressed as g/kg of body weight.

The significance of mean differences was calculated by application of the Student's t test. All error estimates are reported as standard errors (SE).

Results. The mean lung hemoglobin content, expressed as mg/g of lung weight, was 24.3 ± 0.7 in 14 control mice, 23.2 ± 1.4 in 15 mice surviving 30 min of vibration and 28.6 ± 1.5 in 26 mice expiring during vibration. The corresponding values, calculated as g of hemoglobin/kg of body weight, were 0.142 ± 0.005, 0.143 ± 0.007, and 0.207 ± 0.001. In either units, lung hemoglobin content was significantly greater ($p = 0.02$) in the expired than in the other two groups. No significant differences were found between the control mice and the vibrated survivors.

Mean values for lung blood and edema fluid weight in the three groups of mice are given in Table I. The expired group showed a significant increase in lung weight ($p = 0.05$) and in calculated lung blood content ($p = 0.001$) compared to both the control and surviving groups of mice. Surviving mice had significantly heavier lungs than control animals ($p = .05$), but the two groups exhibited no difference in lung blood content.

The calculated amount of edema fluid present in the lungs of vibrated mice compared to control animals was 0.597 and 0.912 g/kg of body weight in surviving and expiring mice, respectively. The difference between these mean values was nonsignificant.

Discussion. In the present study, mice dying during whole-body vibration exhibited a significant increase in pulmonary blood content, compared to sacrificed control animals. No increase in lung blood weight was observed in mice surviving vibration. On the other hand, a similar degree of pulmonary edema was induced in all vibrated mice, whether expiring or surviving. Therefore, vibration lethality appears to be correlated with an increase in pulmonary whole blood content rather than with pulmonary edema. Such conclusions are consistent with previously reported studies in which lungs of mice dying during vibration had large diffuse areas of surface hemorrhage, while lungs in survivors displayed petechial or patchy, rather than diffuse, areas of hemorrhage (3). Similar observations were made in the present investigation. Although such gross observations indicate that much of the increase in lung whole blood seen in the present study, in expired

animals, is the result of hemorrhage, the methods employed do not differentiate between increases in intra- and extravascular blood.

Although the mechanical trauma of vibration undoubtedly contributes in a major way to the lethality observed, a central nervous system component of the pathological consequences of whole-body vibration seems involved, since centrally-acting drugs were shown to alter mortality significantly under such conditions (3). In view of the results of the present study, it appears that an investigation of the effects of such drugs upon lung hemoglobin content of vibrated mice would be pertinent to an elucidation of the mechanism of protection afforded by such agents.

Summary. The lungs of mice, subjected to whole-body vibration of 25 Hz at ± 10*g* for a period of 30 min, as well as of a group of nonvibrated control mice, were excised and lung weight and lung hemoglobin concentration was determined. From these values, the weight of pulmonary blood and water were calculated as indices of pulmonary blood content and edema, respectively. It was found that mice dying during vibration showed a significantly greater amount of blood, but not of edema fluid, than survivors. No difference in lung whole-blood content was found between the surviving mice and control mice. It is concluded that an increase in pulmonary whole blood content is a major factor in vibrational lethality, and that pulmonary edema contributes little to the incidence of death due to whole-body vibration.

1. Roman, J., Wright Air Development Center Tech. Rept. 58-107, Armed Services Technical Information Agency Doc. **AD 151070**, (1958).

2. Schaefer, V. H., Link, H. J., Farrar, J. U., Wiens, D., and Yost, D. H., U. S. Army Med. Res. Lab. Fort Knox, Kentucky, Rept. **390**, (1959).

3. Aston, R. and Roberts, V. L., Arch. Intern. Pharmacodyn. **155**, 289 (1965).

4. Visscher, M. B., Haddy, F. J., and Stephens, G., Pharmacol. Rev. **8**, 389 (1956).

5. Wintrobe, M. M., "Clinical Hematology," 3rd ed., p. 317. Lea and Febiger, Philadelphia, Pennsylvania (1951).

6. Wintrobe, M. M., Folia Haematol. **51**, 32 (1933).

Received Dec. 26, 1968. P.S.E.B.M., 1969, Vol. 131.