Pulmonary Emphysema and Polycythemia Induced in Rats by Forced Swimming.* (25645)

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In chronic pulmonary emphysema there is an inadequate response of erythropoietic activity to low arterial oxygen saturation(1-10). The following experiments show that chronic pulmonary emphysema induced in rats by forced swimming is associated with polycythemia.

Materials and methods. Seventy-three adult male rats of Long-Evans strain weighing between 250 and 325 g were used. Sixty were placed daily in 2 pools (3 x 3 x 2 feet) with water temperature of 22°C. Every day 10 at a time were made to swim to exhaustion. Exhaustion was produced in 15 minutes during the first month, after which the rats could not swim more than 10 minutes. The experiment was continued 90 days when only 29 rats were alive; death of the others was due to drowning. In surviving rats and controls the following studies were performed. 1) *Roentgenogram of chest.* Under deep ether anesthesia, which resulted in marked reduction of respiratory ventilation, one experimental and one control rat were placed over the same film. The roentgenograms prepared for gross and densitometric study were made on Eastman Industrial x-ray film type AA. Exposures were made at target-to-film distance of 1 meter, with approximately 2 seconds at 68 kilovolts, 10 milliamperes. Development was in Eastman Standard X-ray developer 5 minutes at 20°C (Fig. 1). 2) *Densitometry.* Roentgenograms of chest of both experimental and control animals were scanned for differences in light transmission by a spectographic microdensitometer‡ at constant speed of 1 mm/min. The light slit was 100 μ by 0.1 mm. Three parallel paths of scanning were used of lengths between 2.5 and 3.0 cm; the separation between paths was 0.4 cm. The direction of scanning was from caudal to cephalad. Light transmission values were recorded by Leeds and Northrop Speedmax recorder. Before and after scanning of each roentgenogram, both zero and sensitivity settings of the densitometer were controlled. By end of complete scanning operation the drift was less than 1%. 3) *Hematological determinations.* Blood volume was determined by Fe^{59} tagged cell technic. Labeled blood was drawn into a heparinized syringe by cardiac puncture from a donor that had received 10 μc of Fe^{59} intraperitoneally one week before. Blood volume determination was done under ether anesthesia. After injection of 0.5 cc of donor blood through the saphenous vein, 6 minutes were allowed for mixing after which 2 cc of blood were drawn from jugular vein. An aliquot of this blood was used for counting and for hematocrit and hemoglobin determinations. Amount of radioactivity injected was divided by amount of radioactivity/unit of recipient blood to give the assumed blood volume. This, multiplied by the hematocrit, gave total red cell volume. To be more comparable, blood, plasma and red cell volumes were expressed in cc/100 g of body weight. The hematocrit was determined in Wintrobe's tubes, hemoglobin by method of Turner(11). 4) *Histological sections.* Lungs were fixed by injection of 10% formalin intratracheally before opening the chest. Adrenals were carefully dissected under binocular microscope and weighed. Hematoxylin-Eosin stain was used for both lung and adrenal tissues. For lung tissue Gomori's Aldehyde-Fuchsin stain for elastic fiber and Perl's Prussian blue were used.

Results. Determinations were made of percent light transmission by scanning of chest films. Three determinations were made in each intercostal space of right lung from second to eighth rib, and the results compared with those of controls scanned in topographi-

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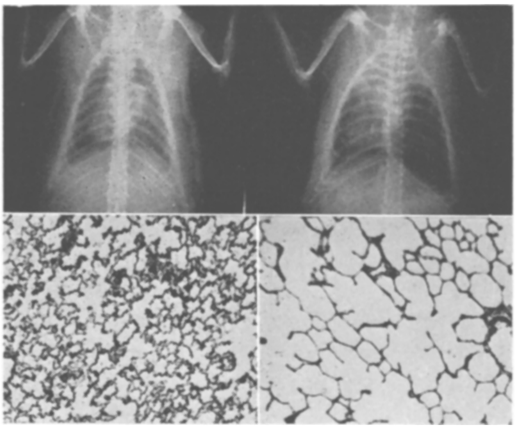


FIG. 1. Chest roentgenogram of control (left) and experimental (right) rats. In the experimental, decrease of density is accompanied by increase of diameter of the rib case. Microphotographs of lungs of same rats are shown below.

cally correspondent places. From Fig. 2 it is evident that the difference in density ranges from a minimum of 15% in seventh intercostal space to a maximum of 24% in fourth intercostal space. The decrease in density obtained from negative films means an increase in amount of air in pulmonary alveoli. This increase is an essential sign for radiological diagnosis of pulmonary emphysema. Total red cell volume was increased 24% in rats that swam (from 2.1 $\pm$ 0.17 cc/100 g to 2.6 $\pm$ 0.22 cc/100 g ($P<0.001$). No change in hematocrit or hemoglobin was observed. Adrenal weight of rats made to swim averaged 44 $\pm$ 1.1 mg compared with 37 $\pm$ 1.8 mg for controls ($P<0.01$). Microscopic examination of lungs of experimental group revealed emphysema which was mild in most cases but marked in a few (Fig. 3), and was characterized by dissolution of alveolar walls with formation of lacunae. Generally alveolar lumens were larger than normal. The elastic fiber stain showed a disruption of fibers and the iron stain showed presence of old hemorrhage.

There was direct correspondence between values from densitometry of chest films and histological changes. The adrenal glands of both groups were histologically similar.

Discussion. Pulmonary emphysema as demonstrated both by roentgenogram of the chest and histological examination of lungs was produced in rats by swimming to exhaustion daily for 90 days. These same rats had a consistent mild polycythemia with average 24% increase in total red cell volume.

It is known(12) that blood volume/g body weight decreases with age in rats (from 2.2 cc/100 g of red cell volume 60-150 days of age to 2.0 cc/100 g after 200 days of age) because the accumulated fat is less well-vascularized than lean tissue. It was possible that exercise prevented deposition of fat and consequently prevented physiological decrease in blood volume expected with age. Body density of control and experimental rats was measured by the method of Siri(13) and the same average values were found for both groups. Since swimming to exhaustion in cold water should provide sufficient stress to stimulate the adrenals(14) and since it is known that increased adrenal activity may produce an increase in circulating erythrocytes(15,16, 17), the adrenals were carefully dissected and weighed. A correlation between increase in adrenal weight and increase in total circulating red cell volume was found (Fig. 4).

The stress of swimming to exhaustion resulted in pulmonary emphysema, increase in total circulating red cell volume and adrenal hypertrophy. The mild polycythemia could be secondary to both increased adrenal activ-

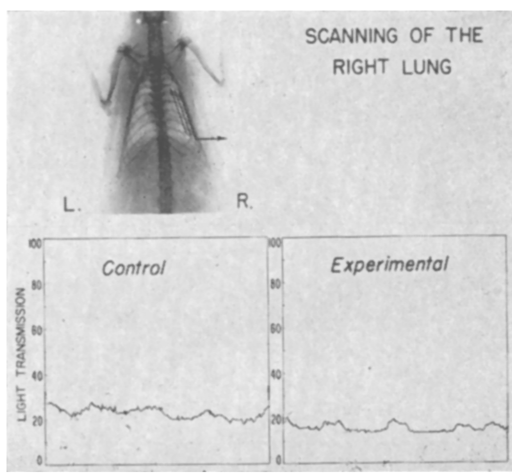
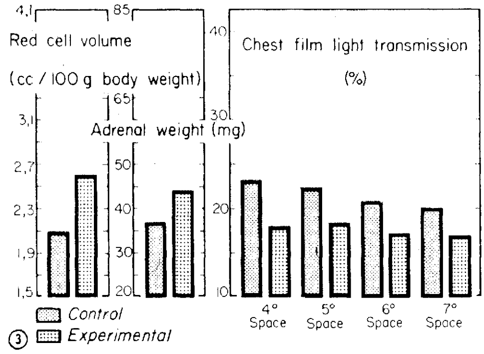


FIG. 2. Densitometry of chest roentgenogram measured by scanning. Photograph shows path followed by light slit of densitometer. Graphs of percent light transmission for control (left) and experimental (right) are shown below. High points on each graph correspond to the ribs.



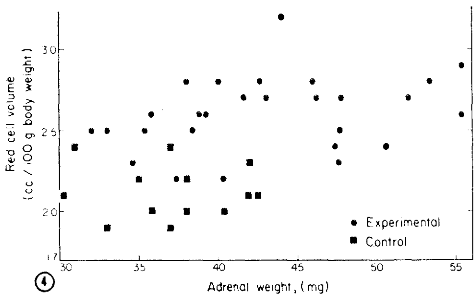


FIG. 3. Red cell vol/100 g B.W., adrenal and % of light transmission obtained by scanning of chest roentgenograms, are compared for both control and experimental groups.

FIG. 4. Shows correlation between red cell vol and adrenal wt.

ity and hypoxia due to pulmonary emphysema. After treatment with ACTH Garcia *et al.*(15) observed an increase of 30% in red cell volume accompanied by 100% increase in adrenal weight in the same strain of rats used in these studies. In the present experiment there was 24% increase in total circulating red cell volume with a relatively small increase in adrenal weight (18%).

This suggests that the degree of adrenal hypertrophy was insufficient to contribute markedly to polycythemia and that the emphysema must have been the primary factor responsible for increase in total circulating red cell volume. This finding is in contrast to the common clinical picture where emphysema is frequently not accompanied by polycythemia.

Summary. Chronic pulmonary emphysema was produced in rats by forcing them to swim to exhaustion each day for 90 days. The emphysematous changes in lungs were accompanied by increase in total circulating red cell volume and adrenal hypertrophy. The mild polycythemia could be secondary both to increased adrenocortical steroid output and hypoxia due to pulmonary emphysema.

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