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Ventricular Weights in Guinea Pigs Acclimated to Cold Plus Hypoxia (42580)

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Abstract. Weanling male guinea pigs (*Cavia porcellus*), 2–3 weeks of age, with initial body weights of 207–271 g were exposed for 2–16 weeks to constant cold (6°C) and hypoxia (P_{O_2} = 85 Torr) equivalent to 4800 m above sea level. Their growth rates and body weights did not differ from those of control animals of the same age maintained under normoxic conditions (22°C, P_{O_2} = 133 Torr). After 2, 3, 4, 6, 10, or 16 weeks exposure the animals were sacrificed, the hearts were removed, the ventricles were separated and weighed, and myoglobin concentrations were determined. Total heart weight as well as both right and left ventricular weights increased linearly with age. By the second week of exposure of the guinea pigs to cold plus hypoxia the total heart and right ventricular weights were 25 and 50% greater than those of the normoxic control animals. Both weights increased at greater rates than those of the controls until Week 6 and then remained at 30 and 80% throughout the 16th week. The weights of the left ventricles in these animals were only slightly greater than those of the controls. In spite of the severe right ventricular hypertrophy these animals showed no clinical signs of right heart failure. Myoglobin concentrations were significantly greater in both ventricles for the cold-plus-hypoxic animals than for the controls.

Mammals at high altitude are affected by cold and hypoxia to varying degrees according to altitude level and geographical latitude (1–4). Despite the low temperatures usually measured at high altitude it has generally been assumed that humans can effectively protect themselves from the cold. In animal studies the effect of cold has largely been disregarded.

Acclimation to high altitude is accompanied by changes in the respiratory and cardiovascular systems (4, 5). During acclimation humans develop increased oxygen-carrying and gas-exchange capacities which allow oxygen consumption to remain essentially unchanged despite lower ambient and tissue P_{O_2}'s. Hartley guinea pigs exposed to ambient hypoxia under normal laboratory conditions develop marked secondary erythrocytosis, increased pulmonary vascular resistance, right ventricular hypertrophy, heart failure, and eventual death (6–8). Hartley guinea pigs raised at 5°C without hypoxia have increased oxygen needs and cardiac output (4, 9) but only a modest erythropoietic response (10). We have further observed that when cold and hypoxia were combined there was a lower oxygen-carrying capacity (11) but no mortality was observed. This occurred despite the increased oxygen needs of the body and higher cardiac output. The present study examines the effects of hypoxia combined with cold on the temporal progression of the weight of the hearts of the guinea pigs to determine whether the degrees of right ventricular hypertrophy and modest left ventricular hypotrophy found in hypoxia at normal laboratory temperatures were affected by exposure to concomitant cold.

Materials and Methods. *Animals.* Weanling male guinea pigs, *Cavia porcellus* (Hartley strain), were obtained from Camm Research Institute, Inc. (Wayne, NJ) at 2 to 3 weeks of age. Weights were 207–271 g upon arrival. Final weights at sacrifice were 253–932 g.

Cold and hypoxia acclimation. Guinea pigs were exposed to cold at 6 ± 0.4°C and hypoxia at an F_{O_2} = 0.134 ± 0.008, which is equal to a P_{O_2} of 85 Torr and equivalent to 4800 m above sea level. The conditions of this experiment have been described by Lechner *et al.* (11) and Banchero *et al.* (12).

Control. Guinea pigs of similar weight and age were kept at an average temperature of 22°C and an average ambient P_{O_2} of 133 Torr in Denver.

Experimental procedures. After 2, 3, 4, 6, 10, or 16 weeks, animals in hypoxia plus cold and age- and weight-matched controls were sacrificed by an overdose of sodium pento-

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barbital. The hearts were removed, the ventricles were separated, the weights of each were determined, and samples for myoglobin concentrations were prepared as described (7).

Covariance analysis and Student's t tests were performed according to Snedecor and Cochran (13). A difference was assumed to be statistically significant when $P < 0.05$.

Results. Guinea pigs exposed to cold plus hypoxia tolerated this stressful environment without any initial losses in body weight and their growth rate was not significantly different from that of control guinea pigs (Fig. 1). Thus, at any given age, the body weights of control and experimental animals were similar. Furthermore, none of the animals exposed to cold + hypoxia showed any clinical signs of right heart failure.

In guinea pigs acclimated to cold + hypoxia as well as in the control animals, total heart weight (THW) and the weight of the right and left ventricles increased linearly with body weight (BW). However, the whole heart and the right ventricles weighed more in the cold + hypoxia animals than those of the corresponding controls. Furthermore, as the BW increased, the THW and right ventricular weight (RVW) increased at a greater rate in the animals in cold + hypoxia. The slopes for THW versus BW and RVW versus BW in the cold + hypoxia animals were significantly greater than those for the control plots (Fig. 2). They were not significantly different for the left ventricle. The values for slopes and

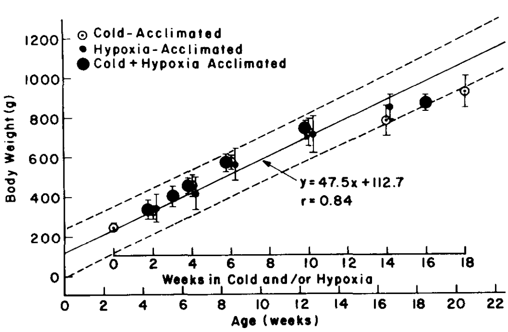

FIG. 1. Relationship between age and body weight in growing guinea pigs. The solid line and equation were determined from data for control animals. The broken lines represent the 95% confidence level. Data for hypoxia- and cold-acclimated animals are included (7). Conditions of the experiments are as described under Materials and Methods.

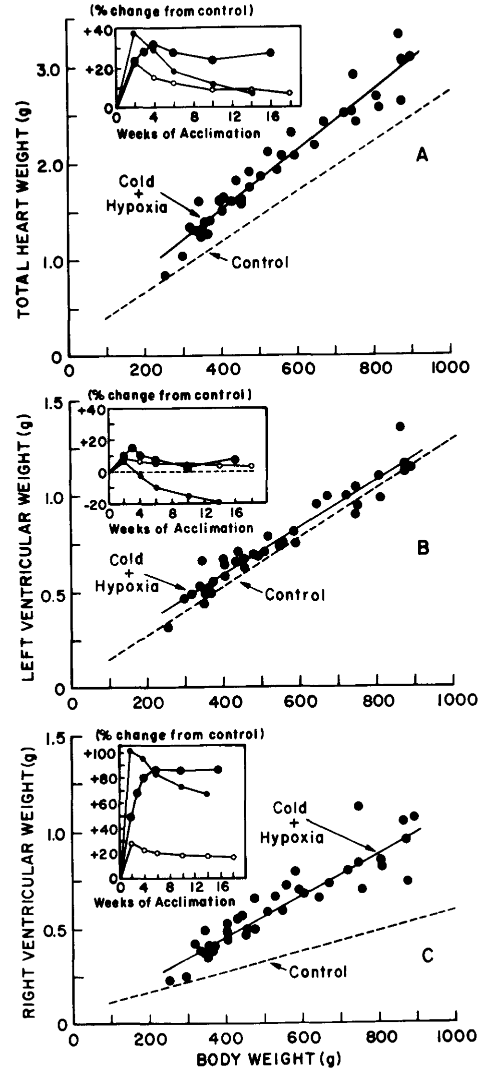

FIG. 2. Total heart weight and ventricular weights in growing guinea pigs acclimated to cold plus hypoxia and in controls. The total heart weight is determined by adding the weight of the right and left ventricles, the right and left atria, and the septum. Each solid circle represents one animal. Inset: The same data (●) were replotted as a percentage change from the values for control animals. For comparison, the data from guinea pigs acclimated to either cold (O) or hypoxia (●) alone are included (7).

intercepts for their regression lines are listed in Table I.

The myoglobin concentration in both the right and the left ventricles was significantly higher in the animals acclimated to cold + hypoxia than in the control group (Fig. 3).

TABLE I. RELATIONSHIP (IN GRAMS) OF TOTAL HEART WEIGHT (THW), RIGHT VENTRICULAR WEIGHT (RVW), AND LEFT VENTRICULAR WEIGHT (LVW) IN GUINEA PIGS UNDER THE EXPERIMENTAL CONDITIONS

	Equations		
	THW =	RVW =	LVW =
This study			
Hypoxia + Cold (n = 38)	$0.288 + (3.11 \times 10^{-3})x$ $r = 0.96$	$0.027 + (1.08 \times 10^{-3})x$ $r = 0.92$	$0.099 + (1.22 \times 10^{-3})x$ $r = 0.92$
Bui and Banchero (7)			
Hypoxia	$0.709 + (2.14 \times 10^{-3})x$ $r = 0.89$	$0.278 + (0.67 \times 10^{-3})x$ $r = 0.65$	$0.214 + (0.08 \times 10^{-3})x$ $r = 0.95$
Cold	$0.428 + (2.45 \times 10^{-3})x$ $r = 0.97$	$0.118 + (0.56 \times 10^{-3})x$ $r = 0.93$	$0.060 + (1.27 \times 10^{-3})x$ $r = 0.98$
Control	$0.165 + (2.58 \times 10^{-3})x$ $r = 0.97$	$0.066 + (0.52 \times 10^{-3})x$ $r = 0.98$	$0.025 + (1.26 \times 10^{-3})x$ $r = 0.97$

Note. The x is the same as body weight in Fig. 2. The r value is the correlation coefficient.

These differences attained statistical significance after the fourth week of exposure. At 4 weeks the average concentrations were 5.15 ± 0.17 and 6.14 ± 0.11 mg myoglobin/g fresh tissue in the right and left ventricles, respectively.

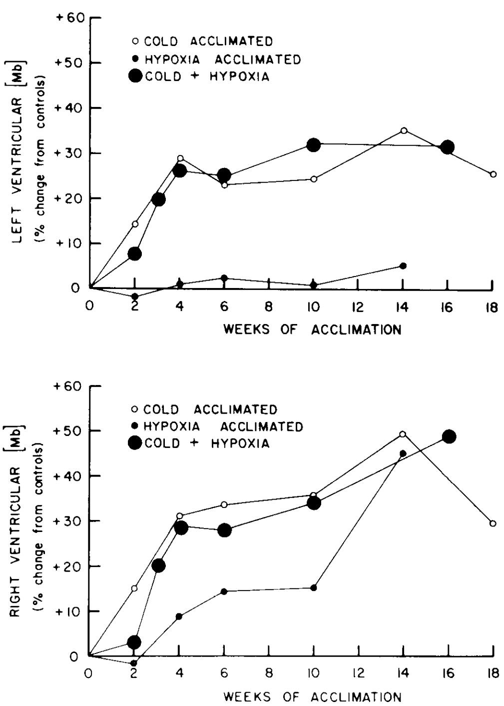

FIG. 3. Changes in myoglobin concentration in the right and left ventricles of guinea pigs acclimated to cold plus hypoxia (●) expressed as a precentage change from the values for the control animals. Data for cold (O)- and hypoxia (•)-acclimated animals are included for comparison (7).

Discussion. It is well recognized that in normoxic animals the increases in body weight associated with normal growth cause a linear increase in total heart weight as well as in the weight of each ventricle (14–17). These normal increases in ventricular weight are caused by myofiber hypertrophy and are accompanied by decreases in capillary density and increases in the capillary-to-fiber ratio as the cross-sectional area of the fiber (FCSA) increases (8, 18). The decrease in myocardial capillary density with growth occurs despite the development of new capillaries (8, 18). Cardiac performance is apparently not affected by this normal age-dependent decrease in capillarity. The literature abounds with data indicating that these growth-induced changes in ventricular weights can be modified by environmental stresses such as cold and hypoxia, especially when these stresses are applied to young, developing animals (8, 18). However, care must be exercised to ascertain if alterations in the weights of the ventricles are indeed related to the superimposed environmental or experimental conditions and are not merely the consequence of a difference in the weight of the animal. We have found in this study as well as in others that the growth rate of the guinea pigs is not affected by exposure to cold or hypoxia if the experimental conditions are applied gradually over a period of days and if water and food are always available to them (11, 12, 19). In all the groups of guinea pigs that we have studied the average body weight for animals of the same age was statistically the same. Therefore, the changes in ventricular weights represent only the effects of the environmental stress (cold and/or hypoxia).

In previous studies we found that cold acclimation of guinea pigs resulted in modest bilateral increases in ventricular weights (Figs. 2B and 2C, insets), whereas hypoxia-acclimated guinea pigs, without cold, developed large increases in right ventricular weight while the left ventricles showed modest decreases in weight (7). Furthermore, Kayar and Banchero (8, 18) have recently shown that the relationship between ventricular weight (macroscopic assessment of hypertrophy) and FCSA (microscopic assessment of hypertrophy) depends on the mechanism causing ventricular growth. In normal myocardial growth there is a proportional increase in FCSA and fiber length (8), and the relationship between FCSA and weight of the myocyte for the right ventricle and the left ventricle is unique (8, 18). In guinea pigs with a pressure overload of the right ventricle (hypoxia acclimated) the increases in right ventricular weights were mainly due to increases in FCSA as it occurs in normal animals (Ref. (8), Fig. 3). But in those guinea pigs with a volume overload (cold acclimated) the increases in left ventricular weights were due to increased fiber length (18). This distinction is important when values of capillary density, capillary-to-fiber ratio, and number of capillaries around the fiber are to be compared to the respective values measured in normal animals, as the relationships for these variables and FCSA are not the same as those to ventricular weight.

Both acclimation to cold and acclimation to hypoxia are accompanied by increases in the oxygen-carrying capacity of blood. The difference in this effect is, however, quite marked under the two conditions, as Lechner *et al.* have shown (6, 10, 11). Two weeks after guinea pigs were exposed to simple hypoxia they showed severe erythrocytosis with hemoglobin concentrations and hematocrit, which were 40–50% higher than those in controls of similar age and body weight (6). The magnitude of the right ventricular hypertrophy in hypoxia-acclimated guinea pigs was partly related to this severe secondary erythrocytosis (8). The guinea pigs acclimated to simple cold showed only modest (10%) initial increases in hemoglobin concentration and hematocrit, which remained at about 5% above controls (10). However, guinea pigs exposed to the two stresses acting concomitantly showed a more gradual erythropoietic response, with hemoglobin concentration and hematocrit increasing 13–15% after 2 to 4 weeks and progressively escalating to 30–35% by 16 weeks of acclimation (11). The guinea pigs acclimated to cold + hypoxia developed a more progressive and stable degree of right ventricular hypertrophy (as judged by increased ventricular weight) than either of the other two groups (Table I and Fig. 2C inset) but showed no mortality due to right heart failure. The more gradual progression of hypertrophy in cold + hypoxia is probably a consequence of the more gradual increase of the hemoglobin concentrations and hematocrit and may be another reason why there was no clinical evidence of right heart failure and no mortality in the cold + hypoxia-acclimated animals despite the presence of severe right ventricular hypertrophy. In fact, the progression of right ventricular hypertrophy was slower in cold + hypoxia than in hypoxia alone and suggests that right ventricular performance was adequate in the cold + hypoxia-acclimated animals.

Exposure of the guinea pigs to cold + hypoxia resulted in increased concentrations of myoglobin in both right and left ventricles. These concentrations were 30–40% greater than the concentrations in the respective ventricle of the normoxic animals and are similar to those previously seen for cold-acclimated animals (7). Myoglobin concentrations were also increased in the right ventricle but not in the left ventricle of hypoxia-acclimated animals. In these animals the stressed conditions resulted in hypertrophy of only the right ventricle. These increases in concentration of myoglobin may represent an adaptive mechanism for delivery of additional oxygen to the myocardium under stress (20, 21).

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