Polycythemia and Right Ventricular Hypertrophy

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The chronic hypoxia of high altitude produces an elevated pulmonary arterial pressure resulting in right ventricular hypertrophy. Courand has proposed that the pulmonary hypertension and subsequent right ventricular hypertrophy found in patients with chronic pulmonary disease are associated with polycythemia, hypervolemia, and increased cardiac output secondary to the hypoxemia present in these individuals. Rotta et al. have suggested similarly that high altitude-induced pulmonary hypertension might be due at least in part to polycythemia with an elevation of the circulating blood volume. More recently Naeye has reported that in response to chronic hypoxia there is a marked thickening of the smooth muscle layer of pulmonary arterioles and an extension of the smooth muscle to some small precapillary vessels that ordinarily do not have a smooth muscle coat. Accordingly Fishman has concluded that the elevated pulmonary arterial pressure associated with the chronic hypoxia of high altitude appears to arise predominantly from the circulation of polycythemic blood through small pulmonary arteries whose walls are thickened by hypertrophic smooth muscle.

It seemed important therefore to examine, separately from the hypoxia of high altitude, the role that polycythemia plays in producing pulmonary arterial hypertension. To this end, mice and rats housed at a barometric pressure equivalent to "sea level" were made polycythemic by the administration of packed erythrocytes and maintained under these conditions for periods up to nine weeks. At the end of this time the weights of their right and left ventricular walls were compared with those from animals exposed to a reduced pressure equivalent to 18,000 feet of altitude in a low pressure chamber for corresponding times, and also with those from control animals. Right ventricular hypertrophy was found in animals receiving intraperitoneal injections of CoCl₂ or intraperitoneal injections of packed erythrocytes, as well as in animals housed in a low pressure chamber.

Methods

Albino rats of the Wistar strain were obtained from Harlan Industries, Cumberland, Indiana.

EXPERIMENT 1

Ten males and ten females received intraperitoneal injections of the strong erythropoietic stimulant CoCl₂ at the rate of 10 mg (2.5 mg elemental cobalt)/kg body wt/day beginning at 65 to 70 and 75 to 80 days of age respectively. Nine males and eight females of comparable age received no injections (untreated controls). To confirm the results obtained, a second group of seven males and eight females, 92 to 97 days of age, were similarly injected with CoCl₂. Six males and eight females served as controls.

EXPERIMENT 2

Eight males, 55 to 60 days old, and eight females, 69 to 74 days old, were exposed in a low pressure chamber to a reduced barometric pressure equivalent to 18,000 feet of altitude for approximately 23 hours/day. Seven males and eight females of comparable age were maintained in the animal room under prevailing normal barometric conditions as controls.

Hematocrits were determined weekly for all experimental animals on blood obtained by clipping the tails while the rats were under light ether anesthesia. Using 1.6% aqueous sodium oxalate as a diluent, van Allen hematocrit tubes were used, centrifuging for 30 minutes at 2750
rpm in a size 1, model SBA (head #240) International centrifuge.

After the experimental animals had received CoCl₂ or had been housed in a low pressure chamber for 63 days, all animals were killed by a blow to the occiput, followed by decapitation. Their hearts were removed and carefully dissected under a binocular dissecting microscope using a pair of fine iridectomy scissors. The great vessels were transected from the heart at the level of the semilunar valves. The paired atria were separated from the ventricles by cutting along the coronary sulcus. Next, a blade of the scissors was placed through the right atrioventricular aperture and a cut was made along the posterior interventricular sulcus to the apex of the heart. Then the tissue between the opening of the pulmonary artery and the right atrioventricular aperture was sectioned. Finally, a cut was made along the anterior interventricular septum, thereby separating the right ventricular wall from the left ventricular wall and the interventricular septum. All tissues were blotted on filter paper and any adhering blood clots were removed with fine forceps. The three samples, paired atrial walls, right ventricular wall, and left ventricular wall, plus interventricular septum, were frozen in wells drilled in a %-inch aluminum plate packed in dry ice. They were lyophilized, dried to constant weight in an oven at 95°C, and weighed on a torsion balance.

**FIGURE 1**
Mean hematocrit curves for 10 male and 10 female rats injected intraperitoneally with 10 mg CoCl₂/kg body wt/day for 63 days.

**FIGURE 2**
Mean hematocrit curves for 8 male and 8 female rats exposed to barometric pressure equivalent to 18,000 feet of altitude for approximately 23 hr/day for 63 days.

**EXPERIMENT 3**
Polycythemia was produced in six female C57BL/6J mice by injecting packed erythrocytes intraperitoneally. From donor C57BL/6J mice, 7 to 8 ml of blood were collected in a calibrated centrifuge tube into which approximately 1 ml of 1.0% heparin in physiological saline had been placed. The whole blood was then diluted with an equal volume of saline and centrifuged for one minute slowly in order to separate out any debris or clots that might have formed. All but the last cubic centimeter of blood was drawn off with a Pasteur pipette, placed in calibrated stoppered centrifuge tube, and centrifuged for 10 minutes at 2000 rpm. The supernatant fluid was aspirated and the packed cells resuspended by adding physiological saline to the 15 ml mark and inverting the tube gently until blood and saline were thoroughly mixed. After the cells were washed in this manner three times, the washed, packed, whole erythrocytes were drawn into a syringe to be injected intraperitoneally into recipient animals at the rate of 0.5 ml/day for four days. Hematocrits were determined and an additional 0.5 ml of washed, packed, whole cells was injected intraperitoneally at six-day intervals to maintain a high hematocrit. Blood for hematocrit determinations was drawn from the orbital sinus into a microhematocrit tube which was spun
at 10,000 rpm for six minutes in a model MB International microcapillary centrifuge. Readings were made on an International microcapillary reader. After 22 days, experimental animals, as well as five noninjected controls, were killed by separating their cervical vertebrae. Their hearts were removed and the right ventricular wall and left ventricular wall plus interventricular septum were dissected and treated in the manner described above.

EXPERIMENT 4

Twenty 4-month-old C57BL/6J mice were made polycythemic as described in experiment 3 and, along with 20 untreated male mice of the same age, were placed in a low pressure chamber from 12:00 noon each day to 8:30 a.m. the following morning at a pressure equivalent to 20,000 feet altitude. Hematocrits were determined for each animal before being placed in the chamber and at weekly intervals thereafter. At the end of one, two, four, and six weeks, members of each group, as well as untreated control animals not exposed to low pressures, were killed and the dry weights of their ventricular walls determined for comparison.

Results and Discussion

An hematocrit curve for the first group of rats injected with CoCl₂ and one for rats housed in a low pressure chamber in experiments 1 and 2 are presented in figures 1 and 2 respectively. The hematocrit curve obtained for the second group of rats injected with CoCl₂ did not differ significantly from that of the first and therefore is not presented. Despite minor differences between rates of erythropoiesis and maximal hematocrit levels attained, the curves presented in figures 1 and 2 are very similar. Figure 3 indicates quantitatively the cardiac hypertrophy developed by rats housed in the altitude chamber. The weight of the whole heart increased significantly, 22.7% for males (P<0.001) and 20.6% for females (P<0.001). When the data for the left ventricle were compared, it was clear that no significant change in weight had occurred. In contrast, the weight of the right ventricle had increased 65.0% in the male (P<0.001) and 81.7% in the female (P<0.001). Hence, a relatively large hypertrophy localized chiefly in the right ventricular wall was associated with a comparatively small total heart hypertrophy.

In figure 4 the data on cardiac hypertrophy...
Comparison of dry weights of whole heart, right ventricular wall, and left ventricular wall of untreated male and female rats with those of rats injected intraperitoneally with 10 mg CoCl₂/kg body wt/day for 63 days. Vertical lines represent ± one standard deviation.

occurring in response to CoCl₂ in two separate groups of animals are presented. In each group a small significant increase in the weight of the whole heart was found; for group 1, females, P < 0.001, males, P < 0.01; for group 2, females, P < 0.05, males, P < 0.02. As in the case of animals housed in the altitude chamber, weights of the left ventricular wall were not significantly different. But the right ventricular walls of animals injected with CoCl₂ were significantly larger when compared with those from control animals; 34.9% and 31.2% heavier for males (P < 0.01 in both cases) and 31.3% and 31.2% heavier for females (P < 0.001 in both cases) in groups 1 and 2 respectively. These data demonstrate that right ventricular hypertrophy is found in animals with polycythemia induced by CoCl₂ as well as in animals housed in a low pressure chamber.

The hematocrit figures for mice in experiment 3, made polycythemic by the injection of washed, packed, whole erythrocytes, are given in figure 5. After an initial hematocrit of 80.2% was obtained, following four consecutive daily injections, the hematocrit fell to 71.0% in 11 days although an additional injection of cells was given on day six. A
Mean hematocrit values for 6 female mice injected intraperitoneally with washed, packed, whole erythrocytes. Point for day zero represents mean value obtained following injection of 0.5 ml packed erythrocytes/day for 4 consecutive days. Additional injections were given on days 6, 12 and 18.

second additional injection was administered on day 12 and a third on day 18 so that by day 22 when the experiment was terminated the hematocrit was 75.6%. The relative dry weights of the right and left ventricular walls of control and transfused mice are presented in figure 6. As in the case of CoCl₂ injected rats, the mean relative dry weight of the right ventricular walls of mice injected with erythrocytes was significantly greater ($P < 0.01$) than that observed in control animals. Unlike the CoCl₂ injected rats, the mean relative dry weight of the left ventricular walls was also greater. A possible explanation for this difference may be that during a slowly developing polycythemia, such as that occurring in CoCl₂ injected rats, time permits vascular channels in the systemic arterial tree to dilate and increase in number by endothelial budding.¹³ Thus the increased blood volume is readily accommodated and peripheral resistance does not rise, systemic arterial pressure is not elevated, and left ventricular hypertrophy does not result. On the contrary, when a severe polycythemia is induced rapidly, as in the transfused mice, perhaps the volume of the systemic vascular tree does not increase quickly enough to compensate for the suddenly increased blood volume so that systemic resistance and blood pressure both increase.

Hematocrit values obtained before and after exposure in a low pressure chamber in experiment 4 are recorded in table 1. After one and two weeks of exposure in a low pressure chamber, hematocrits were substantially higher for mice injected with erythrocytes than for noninjected mice. In figure 7 it may be observed that the relative dry weight of the right ventricular walls was only slightly higher for injected mice at the end of one week. This difference was increased further by the end of the second week but was not statistically significant in either case. Perhaps if two groups of mice, one with a constant high hematocrit and the other with a constant low hematocrit, were maintained at the same low pressure for a longer period of time, the right ventricular...
TABLE 1
Mean Hematocrit Values in Noninjected Mice and in Mice Injected* with Packed Erythrocytes before and after Graded Periods in a Low Pressure Chamber at a Barometric Pressure Equivalent to 20,000 Feet Altitude

<table>
<thead>
<tr>
<th>Time in chamber</th>
<th>Treatment before period in chamber</th>
<th>Hematocrit Before period in chamber</th>
<th>Hematocrit After period in chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>weeks</td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>46.8 (5)†</td>
<td>54.4 (5)‡</td>
</tr>
<tr>
<td></td>
<td>Injected</td>
<td>71.0 (5)</td>
<td>71.0 (5)</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>46.6 (5)</td>
<td>69.0 (4)</td>
</tr>
<tr>
<td></td>
<td>Injected</td>
<td>72.2 (5)</td>
<td>74.2 (5)</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>— (5)</td>
<td>80.5 (4)</td>
</tr>
<tr>
<td></td>
<td>Injected</td>
<td>73.3 (5)</td>
<td>81.3 (3)</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>— (5)</td>
<td>81.4 (5)</td>
</tr>
<tr>
<td></td>
<td>Injected</td>
<td>75.5 (5)</td>
<td>83.0 (2)</td>
</tr>
</tbody>
</table>

* Injected intraperitoneally with 0.5 ml of washed, packed, whole erythrocytes/day for four consecutive days.
† Number of animals in each group placed in the chamber.
‡ Number of animals remaining in each group at end of experiment.

hypertrophy for the group with high hematocrit might indeed become significantly greater than that for the group with the low hematocrit. However, in the present experiment the hematocrits for the two groups of animals reached the same value by the end of the fourth week and the relative right ventricular dry weights were the same. The same relationship held at the end of the sixth week and by this time the relative right ventricular dry weights of both groups of mice were essentially doubled. In contrast, the magnitude of left ventricular hypertrophy was considerably less. Thus, differential right ventricular hypertrophy was clearly established.

It is interesting to compare further, and more carefully, the right ventricular hypertrophy induced by CoCl₂ with that induced by high altitude. If the increase of relative dry weight of the right ventricular wall in CoCl₂-injected rats is compared with that in rats housed in the altitude chamber, the...
former is found to be approximately 45% of the latter, on the basis of pooled data for males and females. Such a direct comparison cannot be made from the data available for mice made polycythemic by the intraperitoneal injection of erythrocytes. However, calculations can provide a reasonable estimate of these values for comparison.

First, the relative dry weights of both the right and left ventricular walls of one of the mice injected intraperitoneally with erythrocytes were very low (fig. 6), so low, in fact, that they do not appear to belong to the same population. The question arises as to whether the body weight could have been recorded incorrectly. This explanation seems unacceptable because the absolute weights obtained for both ventricular chambers of this animal were the lowest recorded for all animals in the experiment, both control and polycythemic. If results for this animal are excluded, the average relative right ventricular dry weight for the remaining five animals receiving intraperitoneal injections of erythrocytes is 23.0 mg, a 3.6 mg increase over the control value.

Second, the mean relative dry weight of the right ventricular walls of noninjected mice exposed in the chamber for two weeks (fig. 7) is 26.6 mg, a 7.4 mg increase over the control value. The corresponding value for animals exposed in the chamber for four weeks is 32.9 mg, a 14.2 mg increase over the control value. If it is assumed that the value for animals exposed in the chamber for three weeks falls midway between the values for animals exposed two and four weeks, then a 10.8 mg increase in relative right ventricular dry weight is estimated. The 3.6 mg increase in mean relative right ventricular dry weight for mice injected with erythrocytes represents 33% of the estimated 10.8 mg increase for animals exposed to high altitude for three weeks. Hence, while it is clear that right ventricular hypertrophy occurred following the injection of CoCl₂ as well as following the injection of erythrocytes, the magnitude of the response was substantially less than that obtained for animals housed in a low pressure chamber.

At autopsy, all experimental rats and mice, whether injected with CoCl₂, injected with erythrocytes, or housed in a low pressure chamber, showed definite signs that an increase in blood volume had occurred. All tissues and organs were a deep red color. Microscopic examination of histological sections of liver and kidney revealed vascular channels dilated and engorged with blood. Therefore, although direct measurements of blood volume were not made, the morphological evidence seems sufficiently strong to warrant the conclusion that a hypervolemia as well as polycythemia occurred in the three groups of experimental animals.

What, then, can be concluded about the role that polycythemia and an associated elevated circulating blood volume play in eliciting right ventricular hypertrophy? Barnard⁷ states that “polycythemia is not of itself a cause of right ventricular hypertrophy.” Valdiva⁶ concluded that the increased blood viscosity caused by polycythemia could not explain right heart hypertrophy because he believed this would affect both the right and the left heart equally. In contrast, the data presented here for mice injected intraperitoneally with erythrocytes provide strong support for the conclusion that a preferential right ventricular hypertrophy, greater than left ventricular hypertrophy, can be induced by polycythemia either with or without hypoxia. The fact that some left ventricular hypertrophy does occur has already been discussed, and may have been due to the rapidity with which the rather severe polycythemia was induced.

It is more difficult to draw conclusions from the data obtained following the injection of CoCl₂, because the mode of action of cobalt is still in doubt. Early workers¹⁴ suggested that cobalt stimulates erythropoiesis by producing an anoxic state in bone marrow. Later experiments¹⁸ showed this suggestion to be untenable. Recent work¹⁸ indicates that cobalt produces respiratory inhibition in liver and skeletal muscle. Therefore,
hypoxia may be the stimulating factor for cobalt-induced polycythemia just as for polycythemia occurring at high altitude. If this is true, then the data presented here suggest that the magnitude of the stimulus for the CoCl₂-injected rats was similar to that for rats housed in an altitude chamber because the rate of erythropoiesis and the maximal hematocrit attained were similar for both groups. On the other hand, the right ventricular hypertrophy obtained in rats receiving CoCl₂ was only 45% of that for animals housed in an altitude chamber. The possibility exists that there was hypertrophy of pulmonary vascular smooth muscle in chamber animals, as described by Rotta et al.¹ and by Naeye,⁹ but not in CoCl₂-injected animals.

Reeves et al.³ produced chronic hypoxia in calves by shunting part of the inferior vena caval blood flow into the left atrium but pulmonary arterial pressure did not increase. However, a rise of pulmonary arterial pressure was produced when these same calves breathed low oxygen mixtures. Reeves et al. concluded that airway hypoxia increased the pulmonary arterial pressure whereas hypoxemia without airway hypoxia did not. If a pulmonary vascular response occurs in altitude animals but not in cobalt-injected rats, then it can be suspected that polycythemia and hypervolemia are the prime factors that initiate pulmonary hypertension in the latter animals. The pulmonary capillaries are unique anatomically because they are located in the extremely thin septa interposed between adjacent air-filled alveoli. It seems possible that the right ventricular hypertrophy which resulted following CoCl₂ administration could have been due to the inability of the pulmonary vascular tree to make the compensatory responses to polycythemia and hypervolemia that the systemic vascular tree was able to make.

The data presented here provide strong support for the conclusion that selective right ventricular hypertrophy can be produced when polycythemia and hypervolemia are present and when airway hypoxia is absent. It is concluded further that polycythemia may be an important factor in the right ventricular hypertrophy that is induced in rats and mice by high altitude.

**Summary**

Polycythemia was induced in rats by injecting 10 mg CoCl₂/kg body wt/day intraperitoneally or by placing them in a low pressure chamber at a reduced barometric pressure equivalent to 18,000 feet of altitude for approximately 23 hours/day. After 63 days of treatment a significant hypertrophy of the right ventricular wall was found in both groups of animals. The increase in weight of the right ventricle in the CoCl₂-injected rats was approximately 45% of that found in rats exposed to chronic hypoxia.

Polycythemia was induced in mice by injecting 0.5 ml of packed, whole erythrocytes per day, intraperitoneally, for four consecutive days. A high hematocrit was maintained by additional periodic intraperitoneal injections of erythrocytes. After twenty-two days a significant right ventricular hypertrophy was observed.

It is concluded that polycythemia and hypervolemia are important factors in producing right ventricular hypertrophy in rats and mice without airway hypoxia and also may be significant factors in the cardiac hypertrophy induced by high altitude.

**Acknowledgment**

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