Effect of Altitude and Cobalt Polycythemia, Hypoxia, and Cortisone on Susceptibility of Rats to Endocarditis

By Benjamin Highman, M.D. and Paul D. Altland, Ph.D.

Rats with polycythemia induced by exposures to simulated high altitudes developed a significantly higher incidence of endocarditis following intravenous bacterial injections than rats with cobalt polycythemia or ground level controls. No increase occurred in altitude rats when polycythemia was prevented by repeated bleedings. These findings suggest that both polycythemia and hypoxia are necessary to explain increased susceptibility of altitude rats. Cortisone increased susceptibility of ground level rats injected with *Streptococcus faecalis*. Unlike altitude exposures, however, cortisone did not render rats susceptible to endocarditis due to *Hemophilus parainfluenzae*.

The susceptibility of rats to bacterial endocarditis can be increased greatly by exposing them discontinuously in a low pressure chamber to a simulated high altitude. The basic causes of such altered resistance are obscure. It is well known that the altitude exposures induce a severe polycythemia, cardiac hypertrophy, altered cardiac output, hypoxia with lowering of arterial pO2, and adrenal cortical hypertrophy. However, the specific role of any one or combination of these factors in the susceptibility to endocarditis has received little attention. We are presenting the findings associated with some of these factors acting individually. Cobalt was used to produce polycythemia without hypoxia, altitude rats were bled repeatedly to study the effect of hypoxia without polycythemia, and cortisone was given to study the possible effect of hyperactivity of the adrenal cortex. The methods used in exposing the animals and in preparing and inoculating the bacteria were similar to those described.

**PATHOLOGY**

The histopathologic changes occurring in the course of experimental endocarditis due to viridans streptococci have been described, but a brief summary will clarify the planning of the experiments and the grading of the lesions.

**Valvular Lesions.** Lesions in altitude rats occurring after a single inoculation of 0.5 cc. of a 6-hour broth culture of *Streptococcus faecalis* may be considered typical. Within 8 to 12 hours, superficial small inflammatory foci appear anywhere along the surface of one or more leaflets. Subsequently, these foci increase in size, are confined chiefly to the mitral and aortic leaflets, and are often surmounted by vegetations containing bacterial colonies. In some cases, the colonies are largely intracellular, lying within macrophages and occasionally within lining endothelial and stroma cells of the leaflet. Bacterial vegetations develop within 20 hours after inoculation, rapidly increase in number and size, and are seen in nearly all rats killed 4 days after inoculation. After four days, the lesions in altitude rats may begin to regress but usually progress and cause death four to 10 days after the inoculation. Comparable lesions are seen in rats given *Streptococcus mutis*. After multiple inoculations the lesions are similar but more severe. The lesions in ground level controls resemble those in altitude rats but are usually milder and seldom fatal; their course simulates that seen in altitude rats treated with penicillin. If therapy of altitude rats is begun 12 or 20 hours after inoculation and before vegetations have formed, resolution without residual changes may be completed within 48 hours. Recovery is less certain and prompt when treatment is delayed for three to five days after large vegetations have formed. In favorable cases, the bacteria may be destroyed by lysis and phagocytosis within 48 hours after beginning therapy, and the inflammatory reaction may subside and the vegetations may be organized within a week. In altitude rats given multiple bacterial inoculations, penicillin therapy may be ineffective, resolution of the lesions may be delayed, and, occasionally, the involved leaflets may show residual thickening and


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other degenerative changes or chronic valvular deformities similar to those seen in man.

Grading of Lesions. Endocarditis was determined by microscopic study of skip serial sections of the heart stained with hematoxylin azure eosin. The lesions were graded severe (black in the bars shown in fig. 1) when sections revealed a severe valvulitis with bacteria in the vegetations. They were graded moderate when sections revealed a valvulitis but no bacteria; generally, these were regressing lesions. Negative findings were recorded when no unequivocal inflammatory lesions were found in the valves; these represented uninfected or recovered rats.

Experiments and Results

The experiments were terminated five or six days after the first bacterial inoculation to minimize mortality; this allowed sufficient time for severe lesions to develop in susceptible animals, while permitting such lesions to begin to regress, but not to disappear, in more resistant animals.

Experiment I. Cobalt was used to determine the effect of polycythemia without general hypoxia on susceptibility to experimental endocarditis. Fifty-eight young male Sprague Dawley rats were divided into the 3 groups shown in the upper portion of figure 1. Altitude group 1 received 12 successive daily 22-hour exposures in a low pressure chamber to a simulated altitude of 18,000 feet before the first bacterial inoculation and daily 4-hour exposures to 25,000 feet for the following 6 days. Cobalt group 2, beginning 143 days before the first inoculation, received a modified stock diet containing in each kilogram at first 500 and later 1000 mg. of cobaltous chloride (CoCl₂·6H₂O); daily food consumption approximated 8 per cent of body weight. Group 3 was maintained at ground level and fed an unmodified stock diet of Purina Laboratory Chow.

All but 4 animals were killed 3 days following the last of 4 successive daily intravenous injections with 0.5 cc of a 6-hour broth culture of Str. mitis JH-26. Severe endocarditis was noted in the 2 altitude and in 1 of the 2 cobalt rats dying before completion of the experiment.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>MEAN HEMATOCRIT</th>
<th>NO. OF RATS</th>
<th>NO WITH ENDOCARDITIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1. ALTITUDE</td>
<td>60</td>
<td>20</td>
<td>8*</td>
</tr>
<tr>
<td>2. COBALT</td>
<td>64</td>
<td>20</td>
<td>8*</td>
</tr>
<tr>
<td>3. GROUND LEVEL</td>
<td>49</td>
<td>19</td>
<td>8*</td>
</tr>
<tr>
<td>4. ALTITUDE (NOT BLED)</td>
<td>73</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>5. ALTITUDE (BLED TWICE)</td>
<td>58</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>3. ALTITUDE (BLED REPEATEDLY)</td>
<td>54</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>4. COBALT</td>
<td>64</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>5. GROUND LEVEL</td>
<td>51</td>
<td>19</td>
<td>2</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of hypoxia and polycythemia on susceptibility to experimental endocarditis. In experiment I, the rats were killed 3 days after 4 successive daily inoculations of Streptococcus mitis, JH-26 excepting 2 cobalt and 2 altitude rats dying from infection. In experiment II, the rats were killed 4 days after 2 inoculations of Str. mitis. Mean hematocrit values were determined shortly before the first bacterial inoculation.

* Incidence is significantly lower (chi-square test) than in Group 1 of the corresponding experiment.
As shown in figure 1, although the hematocrit value of the cobalt group was higher than that of the altitude group, 64 versus 61, the incidence of severe endocarditis was only about half (40 per cent) and nearly the same as in the ground level controls. None of the altitude animals exhibited moderate or regressing lesions.

**Experiment II.** This experiment was designed primarily to determine whether or not hypoxia without polycythemia would increase susceptibility to experimental endocarditis. A series of 113 rats was divided into 5 groups shown in lower portion of figure 1. **Altitude groups 1, 2, and 3** were exposed 4 hours daily to a simulated altitude of 25,000 feet beginning 33 days before the first bacterial inoculation. **Cobalt group 4**, beginning 47 days before the first inoculation, was fed a diet containing in each kilogram 1000 mg. of cobaltous chloride, 600 mg. of ferric chloride (FeCl₃·6H₂O), 40 mg. of manganese sulfate (MnSO₄·4H₂O), and 25 mg. of copper sulfate (CuSO₄·5H₂O). Supplements were added since a high blood building diet is necessary to maintain cobalt polycythemia in the rat.** Ground level group 5** was fed an unmodified stock diet. **Altitude group 3** was bled every 3 to 6 days after beginning altitude exposures to keep the hematocrit value within normal limits and thereby produce a condition of hypoxia without polycythemia. This was accomplished by warming the tail, cutting off its tip and estimating the hematocrit by determining the specific gravity of a drop of freely flowing tail blood by the copper sulfate method.**

**Altitude group 3** was bled three to six days after the first bacterial inoculation to reduce the hematocrit approximately to ground level values. The purpose was to determine whether or not changes in resistance induced by altitude exposures were readily reversible. **Altitude group 1** was not bled and served as a control. All the rats were killed 4 days following 2 successive daily intravenous injections of *H. influenzae* JH-26.

Since only 2, instead of 4 inoculations were given, the incidence of severe endocarditis in this second experiment was low in all groups (fig. 1). However, the total incidence of endocarditis (moderate and severe lesions) in the cobalt group and in the altitude group bled repeatedly was similar to that in the ground level controls and significantly lower than in the altitude controls. The incidence in the altitude group bled twice after polycythemia had developed was intermediate between that in the altitude and ground level controls.

**Experiments with Cortisone:** Since the adrenal gland is hypertrophied in altitude rats, the effect of cortisone was investigated. In one study, one of two groups of ground level rats received 5 mg. per rat of cortisone acetate (Cortone acetate, Merck) twice daily intramuscularly beginning 48 hours before an inoculation of 0.5 cc of a 6-hour culture of *Staphylococcus* aureus. The rats were killed three to six days after the inoculation. As shown in table 1, the incidence of endocarditis (moderate and severe) and of severe lesions was significantly higher in the cortisone group.

In another experiment, cortisone was administered similarly to 1 of 2 altitude groups and 1 of 2 ground level groups (table 1) beginning 3 days before the first of 3 inoculations of *Hemophilus parainfluenzae* 655* (0.5 cc of an 18-hour culture in beef heart infusion broth with dextrose enriched with 2 per cent peptic digest of blood). Survivors were killed 6 days after the first inoculation.

**Table 1.**—Influence of Cortisone Therapy on Incidence of Endocarditis in Ground Level (G.L.) and Altitude (Alt.) Rats

| Group            | No. of Rats | Endocarditis
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Severe</td>
</tr>
<tr>
<td>After 1 I.V. injection <em>St. faecalis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.L. control</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td>G.L. cortisone</td>
<td>28</td>
<td>18</td>
</tr>
<tr>
<td>After 3 I.V. injections <em>H. parainfluenza</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.L. control</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>G.L. cortisone</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Alt. control</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Alt. cortisone</td>
<td>9</td>
<td>4</td>
</tr>
</tbody>
</table>

* Statistically significant by the chi-square test.
† Significantly higher than in ground level rats.

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*Str. faecalis* and *H. parainfluenza* were furnished respectively by Drs. H. Eagle and M. Pittman, National Microbiological Institute.
the incidence of bacterial endocarditis in this study.

**Discussion**

A number of hypotheses were considered in our investigation of susceptibility to endocarditis. In our first reported study, we used rats exposed to a simulated high altitude for three to six months. Such rats often developed, before inoculation, sterile valvular vegetations as well as marked thickening of the leaflets with increased cellularity and increased amounts of collagen, metachromatic material, and material staining for acid mucopolysaccharides. However, in a second study, we found that susceptibility to endocarditis increased immediately after beginning exposures and approached a maximum in 30 days when polycythemia neared its peak and long before the development of sterile vegetations. This indicates that the increased susceptibility is not due to bacterial infection of sterile vegetations.

In the second study, susceptibility of altitude rats to endocarditis seemed to vary with the hematocrit, suggesting that polycythemia was the determining factor. In the current investigation, however, animals with cobalt polycythemia failed to show the increased susceptibility displayed by altitude rats with a lower hematocrit. This indicates that polycythemia alone is not the determining factor. On the other hand, hypoxia also does not appear to be the sole determining factor, since increased susceptibility did not occur in altitude rats when polycythemia was prevented by repeated bleedings. These findings indicate that neither hypoxia nor polycythemia alone suffice to explain the high incidence of endocarditis in altitude rats.

A prolonged increase in cardiac work load has been considered an important factor in increasing susceptibility to endocarditis. For example, Lillehei and his associates have reported a somewhat similar increased susceptibility to endocarditis in dogs after the surgical production of arteriovenous fistulas. They attribute this in part to a mechanical factor pertinent to an enormous increase in the work thrust upon the heart by the arteriovenous fistulas. The marked cardiac hypertrophy noted in our altitude rats indicates that the work load of the heart is increased greatly by the altitude exposures. The lessening of the load on the heart when polycythemia is prevented may explain the increased resistance of the altitude rats bled repeatedly. However, the failure of polycythemia induced by cobalt to increase the susceptibility of rats to endocarditis suggests that a detrimental factor such as hypoxia may be required in addition to overloading of the heart.

Some investigators have suggested that the lowered resistance of altitude rats may be related to hyperactivity of the adrenal gland. Marked hypertrophy of the adrenal gland occurs in both altitude rats and in dogs with arteriovenous fistulas, but was not observed, as indicated in histologic data from an unpublished study, in rats fed a diet containing cobalt for more than a year. Our experiments show that cortisone may indeed increase the incidence of severe valvular infections caused by certain strains of bacteria. However, altitude exposures may render rats susceptible to other bacteria (H. parainfluenzae) the resistance to which is not lowered significantly by cortisone. Roth found that cortisone had little effect on the incidence of endocarditis due to beta-hemolytic streptococci in altitude rats. These findings suggest that cortisone may influence but is not the primary cause of the lowered resistance of altitude rats.

Previous valvular damage has been considered predisposing to bacterial endocarditis. The possibility cannot be ruled out that this may be a factor in altitude rats. The unincubated controls used in this investigation, exposed to altitude for 33 days or less, usually showed no significant valvular changes. However, a few exhibited slight changes, similar to those found after a longer period of exposure, such as a slight increase in acid mucopolysaccharides. Perhaps such changes, however slight, may interfere with the metabolism of stroma cells and endothelium rendering them less able to cope with invading or phagocytosed bacteria.

In this study, when bleeding of altitude rats was delayed until polycythemia had developed,
the incidence of experimental endocarditis was reduced, but not to the level of the ground level controls. Similarly, in a previous study,\textsuperscript{2} when altitude polycythemia was allowed to develop, and then exposures were discontinued after the first of eight bacterial inoculations, the incidence of endocarditis dropped, but not to the level of ground controls. These findings indicate that changes in resistance produced by altitude exposures preceding the bacterial inoculations are only slowly or partially reversible. Further studies are needed to determine whether or not this phenomenon is based on the development and persistence of histochemical changes in the substance of the leaflet.

It may be noteworthy, particularly in planning experiments, that the incidence of endocarditis in ground level controls was lower after eight or twelve bacterial inoculations of \textit{Streptococcus mitis}\textsuperscript{1,2} than after two or four inoculations reported in this study. This is due to the ability of ground level rats, unlike altitude rats, to develop timely resistance and recover despite repeated bacterial inoculations. This difference in developing resistance is particularly well illustrated in our first experiment (fig. 1), in which regressive lesions were noted in a large proportion of the ground level controls but in none of the altitude rats.

The effect of altitude polycythemia on resistance of man to endocarditis has not been studied adequately. However, the high incidence of endocarditis in congenital heart disease,\textsuperscript{17} in which polycythemia may be often associated with hypoxia, suggests that certain factors governing susceptibility to endocarditis in the rat may be applicable, at least in part, to man.

The applicability of our findings to human endocarditis requires clinical investigation. If it is assumed for the moment that the factors in the two species are similar, then perhaps the origin of nonbacterial thrombotic endocarditis in man may in some instances be similar to that of the sterile vegetations seen in altitude rats in which the heart is subjected to prolonged overloading and hypoxia. Our findings also suggest that human bacterial endocarditis may not be uncommon in infections associated with bacteremia, the great majority resolving quickly without residual changes. In infections in which bacteremia may be associated with overloading of the heart and hypoxia or some other detrimental factor (pneumonia or infections in individuals with chronic cardiac or pulmonary conditions), it is possible that bacterial lesions may be more severe and resolution may be incomplete and be responsible for some of the idiopathic chronic valvular deformities later found incidentally at autopsy. Reports in the literature\textsuperscript{12} suggest that the incidence of human bacterial endocarditis has been reduced since the advent of widespread antibiotic therapy. This is consistent with our experimental findings in the rat in which the efficacy of early treatment was demonstrated.\textsuperscript{3}

\textbf{SUMMARY AND CONCLUSIONS}

Rats with polycythemia induced by exposures to simulated high altitudes developed a significantly higher incidence of endocarditis following intravenous bacterial injections than rats with cobalt polycythemia or ground level controls. No increase occurred in altitude rats when polycythemia was prevented by repeated bleedings. Cortisone administration increased the susceptibility of ground level rats to endocarditis following an injection of \textit{Streptococcus faecalis}, but unlike altitude exposures, did not render rats susceptible to endocarditis due to \textit{Hemophilus parainfluenzae}. It is concluded that both polycythemia (increase in work thrust upon the heart) and hypoxia (additional detrimental factor) are necessary to explain the lowered resistance induced by altitude exposures and that excessive production of cortisone is not the primary factor.

\textbf{REFERENCES}


\textsuperscript{3} \textsc{— and Eagle, H.:} Experimental bacterial endo-

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