Effect of High Oxygen Concentrations on Erythropoietin and the Renal Juxtaglomerular Cell.* (28268)

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The problem concerning the exact cellular site of production of renal erythropoietin has yet to be solved. Recently, Osnes suggested the renal juxtaglomerular cell because of the association of high plasma levels of erythropoietin with high degrees of juxtaglomerular granularity in bled and adrenalectomized mice(1). Using similar reasoning, Hirashima and Takaku reached the same conclusion when they noted increases in both juxtaglomerular granularity and plasma erythropoietin in rats made acutely anemic by hemorrhage or phenylhydrazine. They also found decreases in both juxtaglomerular granularity and plasma erythropoietin in rats made polycythemic by transfusion(2). Takaku *et al.* found that with constriction of one renal artery, there were increases in both juxtaglomerular granularity in the ischemic kidney and levels of erythropoietin in the plasma(3). Furthermore, we noted increased erythropoietic activity in saline extracts of kidneys from rats fed a diet low in sodium(4); this diet increases juxtaglomerular granularity(5). Meanwhile, Fisher and Crook had demonstrated that angiotensin has erythropoietic activity(6). The juxtaglomerular apparatus may be related to this observation since it is the primary source of renin(5), a substance necessary for angiotensin production(7).

In a previous publication(8), we suggested that the above experiments failed to separate tissue hypoxia, the basic stimulus for erythropoietin production(9), from changes in the distention of the renal arterial bed, which is felt to be the basic stimulus that regulates juxtaglomerular cell secretion(5). Thus, it was possible that the changes in juxtaglomerular granularity were caused by changes in distention of the renal arterial bed, while, simultaneously, the secretory status of an unknown renal cellular source of erythropoietin was being affected by changes in tissue oxygen tension. Therefore, rats were kept in a chamber containing air at a pressure of one-half atmosphere (equivalent to an altitude of 19,000 feet), thereby providing a low oxygen tension in the tissues without a change in distention of the renal arterial bed. Under these conditions, there was no change in juxtaglomerular granularity; and, therefore, the rate of juxtaglomerular cell secretion was also presumably unchanged. This result seemed to refute the hypothesis that the juxtaglomerular cells secrete erythropoietin in response to hypoxia. Following this, it seemed appropriate to investigate the effect of *hyperoxia* on juxtaglomerular granularity, while at the same time doing nothing which would change the distention of the renal arterial bed. Rats were subjected to an atmosphere of high oxygen concentration in the following manner.

Methods. Fifteen 100 g male Wistar rats were kept in a chamber through which a mixture of 80% oxygen and 20% nitrogen was passed at a rate of 4 to 5 liters per minute. Under these conditions, oxygen concentration in the chamber, as measured by a Beckman oxygen analyzer, remained at 76%. The relative humidity in the chamber ranged from 70 to 100%, and temperature approximated 27°C. Purina laboratory chow and water were given *ad libitum*. The rats were removed from the chamber for about 30 minutes each day while it was being cleaned. During the 21 days in this environment, the rats were able to survive and grow, although growth rate was somewhat slower than normal. A kidney was removed from each of 12 randomly selected rats after 21 days in the high oxygen chamber, and the juxtaglomerular indices of these kidneys were determined according to the method previously described by the Hartrofts(10). Indices were also determined for a group of 12 similar control

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rats fed the same diet and kept for the same period of time at a normal oxygen concentration. The slides in this experiment were stained as a group, masked, and randomly counted by a single observer. Two separate sections from each kidney were scanned, and the average of these 2 sections provided the "true" index for that kidney. This amounted to quantitating the granularity associated with 400 or more glomeruli for each rat.

Results. None of the rats in the *hyperoxic* group showed evidence by gross examination of pulmonary edema or hepatic congestion, as has been previously described in oxygen toxicity(11). The average juxtaglomerular index for the *hyperoxic* group was 27 with a standard deviation of ± 6. The average juxtaglomerular index for the control group was also 27 with a standard deviation of ±10.

Discussion. When animals are subjected to an environment of high oxygen concentration, there is a significant increase in the amount of dissolved oxygen that is carried by the blood(12). Fried *et al.* showed that in rats this situation is associated with markedly reduced plasma levels of erythropoietin (12). Following the line of reasoning previously put forth, if the juxtaglomerular cells were indeed the source of the erythropoietin that is stimulated by hypoxia, one would expect this *hyperoxic* situation to be associated with a decrease in rate of juxtaglomerular secretion. This is usually associated with a decrease in juxtaglomerular granularity(5). However, in our experiment there was no such decrease in the granularity of the juxtaglomerular cells in the *hyperoxic* rats and, therefore, presumably no decrease in their rate of secretion. Thus, the results suggest that the juxtaglomerular cell is not a source of the type of erythropoietin which is regulated by changes in oxygen tension. However, one can not preclude the possibility that the juxtaglomerular cells secrete some other type of erythropoietic substance, but do so in response to a change in distention of the renal arterial bed rather than in response to a change in tissue oxygen tension.

Summary. The relationship of renal erythropoietin to the juxtaglomerular cell was studied. Several investigators have noted a correlation between juxtaglomerular granularity and plasma levels of erythropoietin in the presence of simultaneous changes in distention of the renal arterial bed and in oxygen tension of the tissues. To separate these 2 stimuli, rats were kept in an atmosphere of high oxygen concentration and, thus subjected to *hyperoxia* without obvious changes in the distention of the renal arterial bed. Under these circumstances, during which plasma levels of erythropoietin are known to be low, a decrease in juxtaglomerular granularity did not occur. The results suggest that the renal juxtaglomerular cell does not secrete the erythropoietin which is responsive to changes in tissue oxygen tension.

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