The Interrelationship of Hypoxia, Erythropoietin, and the Renal Juxtaglomerular Cell.* (27839)

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The argument concerning the exact cellular site of production of renal erythropoietin remains unsettled. Recently, attention has been focused on the juxtaglomerular apparatus. Osnes noted high plasma levels of erythropoietin in bled and adrenalectomized mice, and because these situations are also associated with high degrees of juxtaglomerular granularity, he suggested that the juxtaglomerular apparatus was a source of renal erythropoietin(1). Hirashima and Takaku(2) noted a similar association between increases in juxtaglomerular granularity and elevated plasma erythropoietin in rats made anemic by acute hemorrhage or injections of phenylhydrazine. Conversely, both juxtaglomerular granularity and erythropoietin levels were decreased below normal in rats made polycythemic by transfusion. Takaku *et al.* also found in rats that constriction of one renal artery was associated with increased levels of plasma erythropoietin(3); this procedure increases juxtaglomerular granularity in the kidney whose artery is constricted(4). Fisher and Crook demonstrated that angiotensin stimulated erythropoiesis in hypophysectomized, adrenalectomized rats(5). The juxtaglomerular apparatus may be related to this observation since it is the primary source of renin(8), a substance which is necessary for angiotensin production(6). Finally, in our laboratory, we found increased erythropoietic activity in saline extracts of kidneys from rats fed a diet low in sodium; this diet increases juxtaglomerular granularity(4,7).

In all of the above experiments, the stimulus of a change in distention of the renal arterial bed has not been clearly separated from that of a change in oxygen tension of the renal parenchyma. The concept that the juxtaglomerular cell is a stretch receptor, sensitive to changes in distention of the renal arterial bed is not new(8). Thus, the increase in juxtaglomerular granularity associated with acute blood loss, adrenalectomy, a low sodium diet, or constriction of the renal artery is thought to represent an increased rate of secretion in response to underdistention of the renal arterial bed(4,8,9). Conversely, the decrease in granularity associated with various types of salt loading is thought to represent decreased secretion in response to overdistention of the renal arterial bed(8).

Hypoxia is now considered to be the basic stimulus for elaboration of erythropoietin (10). In a situation of acute decrease in arterial blood volume, whether due to hemorrhage, adrenalectomy, or hemolysis, both hypoxia and hypovolemia coexist. Similar conditions exist locally in the kidney after its artery has been constricted. Conversely, polycythemia is associated with both hypervolemia and hyperoxia. Thus, most of the above studies may be measuring the simultaneous effects of changes in distention of the renal arterial bed on juxtaglomerular secretion and of changes in renal oxygen tension on the secretion of erythropoietin by some other kidney cells.

In an attempt to separate these stimuli, we studied juxtaglomerular granularity in rats rendered severely hypoxic by being kept in a chamber at one-half atmospheric pressure for 12 hours. This is equivalent to an altitude of approximately 19,000 feet above sea level (11). We elected to determine juxtaglomerular granularity after 12 hours of hypoxia because: First, the level of plasma erythropoietin is high at this time(12,13); second, reticulocytosis has not yet occurred(14) so that the volume of circulating cells is probably unchanged; third, a proven strong stimulus of juxtaglomerular cell secretion, sudden drastic dietary sodium restriction in young rats, has been shown to cause a maximal acute juxtaglomerular degranulation at 12 hours, after

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which the granularity increases to levels above normal(15). Thus, if the juxtaglomerular cells were indeed the source of erythropoietin or its precursor, one would expect to see a decrease in their granularity after 12 hours of severe hypoxia.

Methods. Male Wistar rats weighing 100 g were used. Twelve rats were kept in a chamber maintained at one-half atmospheric pressure by means of a vacuum pump and a regulatory valve. Purina laboratory chow and water were given *ad libitum*. After 12 hours of sustained hypoxia, a kidney was removed from each rat. The kidneys were stained and the juxtaglomerular indices were determined according to the method previously described by the Hartrofts(7). Two separate sections from each kidney were examined, and the average of these 2 sections provided the "true" index for the kidney. This amounted to quantitating the granularity associated with 400 or more glomeruli for each rat. Indices were also determined for a control group of 12 rats fed the same diet and kept at normal atmospheric pressure. All the sections were examined by one observer. Moreover, each of the slides was masked during its examination so that its origin was completely unknown to the observer.

Results. The average juxtaglomerular index for the hypoxic group was 24 with a standard deviation of $\pm$ 9.4. The average index for the control group was also 24 with a standard deviation of $\pm$ 7.4.

Discussion. The results demonstrate that young rats subjected to 12 hours of hypoxia undergo no change in their juxtaglomerular granularity, and therefore, the rate of juxtaglomerular secretion is probably unchanged. One might interpret these results as evidence that renal erythropoietin is not produced by the juxtaglomerular cell. However, one might alternatively postulate the existence of 2 separate renal erythropoietic substances, one of which is secreted in response to underdistention of the renal arterial bed, and the other in response to hypoxia. The former type of erythropoietin could conceivably arise from the juxtaglomerular cell and would perform the useful function of stimulation of erythrocyte production in response to the stimulus of arterial underdistention. The second erythropoietic substance, elaborated in response to hypoxia, might be produced by another, as yet undesignated, renal cell. With this biologic arrangement, a purely hypoxic stimulus would not be expected to cause changes in juxtaglomerular granularity.

Summary. The relationship of renal erythropoietin to the juxtaglomerular cell was studied. Several investigators have noted the frequent association of increased juxtaglomerular granularity and increased levels of plasma erythropoietin after the combined stimuli of underdistention of the renal arterial bed and hypoxia of the renal parenchyma. To separate these two stimuli, rats were subjected to hypoxia without hypovolemia by being kept at one-half atmospheric pressure for 12 hours. At the end of the hypoxic period, there was no change in the granularity of the juxtaglomerular cells and, therefore, probably no change in their rate of secretion. Two hypotheses concerning the cellular source of renal erythropoietin are presented.

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