EFFECTS OF HIGH ALTITUDE ON GRANULAR JUXTAGLOMERULAR CELLS AND THEIR POSSIBLE ROLE IN ERYTHROPOIETIN PRODUCTION

HARRY B. DEMOPOULOS, M.D., BENJAMIN HIGHMAN, M.D.
PAUL D. ALTLAND, PH.D., MARTHA A. GERVING,* AND GABOR KALEY, PH.D.

From the Laboratories of Experimental Pathology, and Physical Biology, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md., and the Departments of Pathology, University of Southern California School of Medicine, Los Angeles, Calif., and New York University School of Medicine, New York, N.Y.

The granular juxtaglomerular (j.g.) cells are modified smooth muscle cells that comprise part of the media of the afferent renal arteriole.[1, 2] The production of renin is an established endocrine function of these cells.[3, 4] The proteolytic enzyme, renin, acts on a plasma globulin, resulting in angiotensin, a potent pressor which also stimulates production and release of aldosterone.[5, 6]

The stimuli responsible for increasing the secretory activity of j.g. cells generally cause a decrease in renal blood flow or in the volume of the renal vascular bed, i.e., renal hypovolemia.[7, 8] Accordingly, partial constriction of the renal artery, narrowing of the suprarenal aorta or oligemic shock are associated with an increase in secretion. The decreased pressure or stretch exerted on the j.g. cells is thought to serve as the actual stimulus for their activity.[8] Overdistention of the renal vascular bed, as by transfusion polycythemia, or a high head of perfusion pressure, decreases secretion.[7, 8] Sodium deficiency increases, while salt loading decreases their secretion.[8, 9] This data is based largely on parallel studies of the renal renin content and histologic appraisals of the secretory state in j.g. cells.

Lowered renal cortical oxygenation may occur with some, or most of the known stimuli that increase j.g. secretory activity. Exactly what role, if any, renal hypoxia plays in j.g. stimulation is not known. In order to examine this parameter alone, renal hypovolemia must be avoided. The degree of hypoxia must not be so severe as to cause relaxation of vascular smooth muscle, nor should the oxygen tension be so low that cellular secretory functions cease. Negative results have altered the use of carbon monoxide in the study of j.g. cells in the hypoxic state.[10] This may be related to the severity of carbon monoxide-induced hypoxia;

Work was initiated and conducted in part while the senior author was at the National Institutes of Health, Bethesda, Md. Dr. Demopoulos' present address is the University of Southern California School of Medicine, Los Angeles, Calif.

Accepted for publication, September 9, 1964.

this agent not only ties up oxygen binding sites on hemoglobin, it also inhibits the release of bound oxygen from hemoglobin.[11]

In the present work the altitude and duration of exposures, as well as the age and sex of the rats selected, were known to result in polycythemia with a very low mortality rate. Furthermore, it was known that neither systemic blood pressure, nor renal blood flow would be diminished during this schedule.[12] In addition to permitting an evaluation of hypoxic states on j.g. cells, the altitude experiments allowed investigation of the relationship between these cells and erythropoietin production.

Erythropoietin (ESF) is a humoral agent that appears in the blood in detectable levels most commonly in response to hypoxia.[13, 14] It may be a sialic acid-containing glycoprotein produced largely by the kidney,[15, 16] the cortex rather than the medulla having been implicated in its production.[17] A relationship between j.g. cells and ESF has been sought by others,[18, 19] but unfortunately the stimuli used to induce hypoxia have also caused renal hypovolemia. Therefore, the observed hyperplasia of j.g. cells accompanying high levels of plasma ESF could have been fortuitous — the j.g. cells having responded to hypovolemia, and the ESF appearing as a result of the effects of hypoxia on other cells.

In the past, a major problem in delineating a valid relationship between j.g. cells and ESF has been the inability to extract active ESF from the kidneys readily.[20, 21] A partial solution to this problem has been obtained in the present experiments, and has permitted a closer correlation between j.g. cells and ESF.

Material and Methods

Bioassay of Erythropoietin (ESF). This was determined by Stohlman's method.[22] The percent of a known amount of Fe^{59} which is incorporated into red cells of starved rats following 2 daily injections of the assay material is used as an expression of the ESF content of the material. Plasma as well as tissue extracts were analyzed by this method. Every sample was assayed simultaneously in 5 rats. The volume of injected material was always 1 per cent of the starved rat's body weight.

Histologic Methods and Evaluation of Juxtaglomerular (j.g.) Cells. Kidneys fixed in Helly's fluid were sectioned and stained with Bowie's stain.[9] The granular j.g. cells were enumerated by a technique that quantitates their number and granularity sufficiently well for biologic comparisons.[23] The number obtained is the juxtaglomerular index (J.G.I.). Other organs, including heart, lung, liver, spleen, adrenal, stomach, duodenum, cecum, ileum, pancreas, testis, bone, skeletal muscle and a portion of each kidney were fixed in 10 per cent buffered formalin and sections were stained with hematoxylin and eosin. In some instances the alcian blue stain and the periodic acid Schiff reagent were utilized to evaluate changes in tissue mucopolysaccharides.

Altitude Studies. A chronic intermittent schedule of exposures in altitude chambers, known to induce polycythemia in rats, was used. Sprague Dawley rats, approximately 200 gm each, and between 80 and 90 days in age, were exposed to "25,000 feet" for 6 hours per day, 5 days per week, for 6 weeks, the rate of "climb" being 2,000 feet per minute.[24] The animals were housed in the chambers within cages

that assured adequate ventilation. Temperature was maintained at approximately 22° C, and Purina lab chow and tap water were given *ad libitum*. Animal weights were individually recorded.

At the conclusion of each experimental situation the rats were bled by aortic puncture and the organs quickly removed. Aliquots of blood were taken for hematocrit determination and then the plasma was promptly separated and stored frozen until assayed for its erythropoietin content. In general there were 4 to 6 rats in each experimental group, and their plasma was pooled in order to have sufficient volume for the bioassay. Sodium levels were determined on aliquots of the various plasma specimens by flame photometry. Blood volume was determined in some of the rats by dilution of radio-chromium tagged red cells. Rats of the same strain, sex, age and weight kept at normal atmospheric pressure served as controls.

In acute experiments the effects on j.g. cells and ESF of 1 and 2 exposures at 30,000 feet were studied on a group of similar rats.

Deoxycorticosterone Hypertension and Salt Loading Experiments. Thirty Sprague-Dawley female rats each weighing about 110 gm were subjected to unilateral nephrectomy; a 25 mg pellet of deoxycorticosterone acetate was implanted subcutaneously 4 days after the nephrectomy. They ate Purina lab chow, but a 1 per cent NaCl solution replaced tap water for drinking. Thirty untouched rats of the same strain, sex and weight were given a 3 per cent NaCl solution to drink, while an equal number of control rats had ordinary tap water. Purina lab chow was fed *ad libitum* to all 3 groups. After 22 weeks on these regimens the animals were sacrificed and the organs promptly removed and then processed for histologic examination and assay of tissue erythropoietin content.

Aqueous extracts of renal cortices and livers were prepared by first homogenizing the freshly excised tissues in the cold, 2 gm tissue per ml of triply distilled H_2O. The whole homogenates were incubated at 37° C in closed vessels under a variety of conditions including different overlying gaseous atmospheres, and the presence or absence of plasma. The whole homogenates were then centrifuged at 4,000 × G for 20 minutes at 5° C; the supernate was then assayed for erythropoietin activity.

Results

Chronic intermittent exposures to simulated high altitudes for a period of 6 weeks resulted in marked polycythemia and expansion of the total blood volume. During the development of polycythemia, rats were sacrificed at periodic intervals; some just before, others immediately after, an exposure in order to study any parallel changes in the juxtaglomerular (j.g.) cells and the circulating erythropoietin (ESF) levels. The results are summarized in Table I. In order to decrease the mortality rate during the initial periods of this schedule the first exposure was at 18,000 feet, whereas all subsequent ones were at 25,000 feet.

In the first week, 3 successive exposures elevated the average J.G.I. (Figs. 1 and 2). The amount of ESF liberated into the circulation at this time was high, causing 24 per cent of the injected Fe^{59} to be incorporated into the circulating red cells of the assay rats. By the end of the first 5 exposures on Friday the juxtaglomerular index dropped below its high mid-week level of 67, but remained well above normal. The Friday circulating ESF level of 14 per cent was considerably lower than Wednesday's 24 per cent.

TABLE I

EFFECTS OF CHRONIC INTERMITTENT EXPOSURE TO SIMULATED HIGH ALTITUDES *

Control values		First week		Sat.	Second week		Sat.	Third week			Sat.	Fourth week
		Wed.	Fri.	Sun.	Mon.	Fri.	Sun.	Mon.	Tues.	Fri.	Sun.	Fri.
J.G.I. †	Pre-exposure	67 (54-83)			61 (51-73)	54 (43-58)		58 (47-65)	62 (56-69)	51 (47-62)		
36 (28-40)	Post-exposure	54 (40-61)	49 (45-57)		50 (37-53)	48 (36-51)		43 (38-54)	46 (35-58)	43 (26-49)		47 (34-50)
ESF ‡	Pre-exposure				5%					6%		
4%	Post-exposure	21%	14%		26%	16%		25%	23%	14%		15%
Blood volume § 6.7%						8.2%				9.6%		
Hematocrit 45	Pre-exposure				52			61		64		
	Post-exposure	45	49		52	57		60		65		68
Serum sodium 137	Pre-exposure			136					140			
	Post-exposure	134						138				

* First day exposed to 18,000 feet, all others at 25,000 feet. Duration of exposures: 6 hours per day, 5 days per week for 6 weeks with normal altitudes on Saturdays and Sundays. Only the first 4 weeks of the 6 week regimen are shown.

† J.G.I. = juxtaglomerular index. The average J.G.I. and range for each interval are shown. Each group of figures represents 4 to 6 rats.

‡ ESF = Erythropoietic stimulating factor. The results are in terms of percent of a known amount of injected Fe^{59} incorporated into circulating red cells of assay rats following injection of plasma from exposed rats. Each value shown represents the average from 5 assay rats.

§ Blood volume is shown in terms of percent of body weight.

After a weekend of rest the J.G.I. on Monday, prior to exposure, was 61, and then 50 following the 6-hour exposure. A correspondingly large amount of ESF was present in the plasma, causing a 26 per cent incorporation of Fe^{59} in the assay rat. On Friday of the second week the difference in the J.G.I. pre- and post-exposure, was small, from 54 down to 48, respectively; the parallel circulating ESF level after exposure on this day was 16 per cent. The J.G.I. and ESF levels behaved in the same manner during the third week. Large decreases in the J.G.I. occurred after exposure early in the week and were accompanied by high plasma erythropoietin levels. Slight drops were observed in the J.G.I. post-exposure later in the week, which were correlated with low levels of plasma ESF.

The increased secretory activity of j.g. cells occurred in the face of an expansion of total blood volume, and in the presence of normal plasma sodium levels (Table I). The viscera, especially the kidneys, had an increasingly plethoric appearance as the hematocrit rose. Microscopically, renal glomerular capillary congestion paralleled the hematocrit value.

Examination of other organs from the rats sacrificed at the intervals shown in Table I failed to reveal histologic evidence of increased secretory activity by other groups of cells. There were, however, some microscopic alterations in structure. Focal areas of fatty change in skeletal muscle, increased cellularity of marrow, and focal degeneration of seminiferous tubular cells were among the changes observed.

In the acute hypoxia studies (Table II) normal rats exposed once or twice to a simulated altitude of 30,000 feet showed a lowering of the

TABLE II

ACUTE ALTITUDE STUDIES *

Control Values	A	B	C
	1st 6-hour exposure	1st 6-hour exposure 18 hours rest 2nd 6-hour exposure	Same as B plus 18 hours rest
J.G.I.†			
39	30	33	48
(28-41)	(22-44)	(19-43)	(39-55)
ESF ‡			
4%	19.1%	16.2%	5.2%

* Exposed to 30,000 feet.

† The average and range of juxtaglomerular indices are shown.

‡ The per cent of Fe^{59} incorporated into red cells of assay rats, following injection of exposed rats' plasma is shown. Values represent the average from five assay rats.

J.G.I. immediately after exposure. When an 18-hour period of rest was added before sacrifice, however, the average J.G.I. rose to 48, significantly above normal.

Erythropoietin was readily extracted from the normal rat renal cortex but anaerobic incubation was a prerequisite (Table III). Supernates from whole homogenates, which were incubated under 95 per cent N_2: 5 per cent CO_2 at 37° C for 1 hour, contained significant amounts of ESF. Serum added to aerobically incubated homogenates failed to influence

TABLE III

CORRELATION OF TISSUE ERYTHROPOIETIN CONTENT AND JUXTAGLOMERULAR INDICES

Extracts	Homogenates * incubated in air		Homogenates † incubated anaerobically		
	Supernate ‡ alone	Supernate § plus serum	Supernate alone	Supernate plus sialidase	J.G.I.**
Normal renal cortex	6% ††	4%	16%	5%	37 (24-51)
DOCA hypertensive-renal cortex	5	5	4	—	3 (0-5)
3% salt loaded renal cortex	6	4	6	—	8 (0-11)
Normal liver	4	6	5	—	—

* Incubated at 37° C, for 1 hour.

† Incubated at 37° C, for one hour; overlying gas phase 95% N_2: 5% CO_2.

‡ Volume of supernate injected was always 1% of assay rats body weight.

§ Serum obtained from same rats whose kidneys were extracted. Final concentration of serum was 5%.

** The average juxtaglomerular index and range are given.

†† These values represent the per cent of Fe^{59} incorporated into circulating red cells of assay rats following injection of the various supernates. The averages from five assay rats are shown for each group.

the amount of ESF in the supernate. Sialidase[15] inactivated the erythropoietin extracted as a result of anaerobic incubation of normal renal homogenates. The deoxycorticosterone and salt loading regimens caused a marked drop in the number and granularity of j.g. cells. The average J.G.I.'s were 4 and 2, respectively (Table III). Extracts prepared from such kidneys did not contain measurable amounts of ESF. The correlations between granular j.g. cells and extractable tissue erythropoietin are summarized in Table III. Hematoxylin and eosin stained sections of these kidneys failed to reveal the tubular epithelial vacuolization characteristic of potassium deficiency.

DISCUSSION

The foregoing data indicates that hypoxia, unaccompanied by renal hypovolemia, results in a stimulation of the secretory activities of the

granular j.g. cells. These experiments have also shown a distinct correlation between the granularity of the j.g. cells and the level of circulating, and extractable renal erythropoietin.

During the 25,000 feet exposures there were no episodes of renal hypovolemia. It has been shown by other investigators that during such stimulation the blood pressure rises slightly and renal blood flow does not decrease.[12] After exposure, these hemodynamic parameters return to normal. More severe episodes of hypoxia, such as those induced by carbon monoxide poisoning, do cause a marked decrease in renal blood flow. Severe hypoxia of this degree, however, should not be equated with that induced at a simulated altitude of 25,000 feet.[11] Dehydration apparently did not occur to any significant extent during exposure as evidenced by the lack of change in hematocrit values before and after exposure (Table I). In spite of the absence of renal hypovolemia, the J.G.I. increased. This rise could not be attributed to alterations in plasma sodium levels either (Table I). The most prominent aspect of the exposures was hypoxia and it seems, for the above reasons, to be valid to suggest that the j.g. cells were stimulated by this factor alone.

Whether the hypoxia, that may occur with some of the usual hypovolemic j.g. cell stimuli (renal artery constriction, shock, etc.), is responsible for the increased secretory activity of these cells can not be clarified by the foregoing experiments. It may well be that the j.g. cells respond to several types of stimuli, hypovolemia, hypoxia, and sodium deficiency.

In assessing the endocrine functions of the granular j.g. cells the relative rates of production, storage and release of granules must be considered. Depending on its intensity, duration and method of application, a secretory stimulus may have varying effects on the 3 secretory steps and the J.G.I., which is the result of a balance among the 3. For instance, within a few hours following a first sodium deficient meal the J.G.I. drops. If the diet is prolonged over several weeks, however, the J.G.I. rises very high above normal.[25]

Correlating the three separate steps in cellular secretion with levels of a circulating or extractable hormone permits a more valid analysis of the cells' endocrine activities. It is not possible to correlate a secretory release of j.g. granules with the simultaneous appearance of a circulating humoral agent if a constant stimulus has been used. The usual j.g. cell secretory stimuli such as ligation of a renal artery or a prolonged sodium deficient diet result in a fixed granularity of the j.g. cells. By its intermittent nature the hypoxic-polycythemic schedule used in the present work facilitated an intimate correlation of production, storage and release of j.g. granules with circulating levels of erythropoietin. This was best demonstrated by the pre- and post-exposure values.

On Monday of the second week (Table I) the pre-exposure J.G.I. was

high and plasma ESF was not detectable. Following the hypoxic stimulation a significant drop occurred in the J.G.I. accompanied by high plasma ESF levels. Furthermore, the magnitude of the daily drop in the J.G.I. paralleled the amount of erythropoietin released. On Friday of this week for example, the J.G.I. decreased to a lesser extent than on Monday, and the amount of ESF liberated was likewise less.

The differences between Mondays and Fridays in the same week, in J.G.I. and ESF, are interpreted as exhaustion phenomena. Five successive exposures to a simulated altitude of 25,000 feet is a rigorous schedule. It is known that continuous and severe hypoxia, e.g. 19,000 feet for 48 hours,[14] results in a cessation of ESF elaboration. This exhaustion aspect of ESF production fits well with the observed differences in the Monday and Friday pre-exposure J.G.I. levels and the lower amounts of ESF liberated at the end of the week on Fridays (Table I).

The acute, 30,000 feet exposures (Table II) were designed to show what a very severe stimulus might do. Goldfarb and Tobian[26] attempted to show that j.g. cell granularity was not related to ESF elaboration on the basis of similar acute altitude studies. They reasoned that if j.g. cells did produce this material then an acute stimulus, known to release ESF, should be accompanied by a lower J.G.I. at its conclusion, demonstrating secretory release of the granules. The simulated altitude used, 19,000 feet, released ESF, but did not affect the J.G.I. In the present work a higher altitude was employed, 30,000 feet. Immediately following this the J.G.I. was considerably lower than in matched controls kept at normal atmospheric pressure (Table II), suggesting a related secretory release of j.g. granules.

The correlations between the extractable renal erythropoietin and the J.G.I. (Table III) are in harmony with the data derived from the altitude exposures. The DOCA and salt loading regimens are reliable methods for decreasing the number and granularity of j.g. cells. The purpose of this series of experiments was to simply show any relationship between the number of granular j.g. cells and the amount of extractable renal erythropoietin. These regimens have far reaching systemic effects and may also cause other changes in the kidneys, such as tubular epithelial vacuolization secondary to potassium deficiency. Damage to other renal cells could, therefore, have been incriminated in the lowering of the ESF content of DOCA and salt-loaded kidneys, but on the basis of careful histologic examination few or no changes consistent with potassium deficiency were seen. The only regularly observed changes in these kidneys, in addition to depletion of j.g. granulated cells, was minimal hyalinization of some afferent arterioles.

Why anaerobic incubation was necessary in the extraction of erythro-

poietin from renal cortices is not clear. It could be related, however, to the sialic acid moiety of ESF. In tissues, sialic acid may be bound to a basic grouping of another substance, possibly a basic protein. If this is the case then hydrolysis of such a bond may be required to release ESF in its active form. It may well be that anaerobic incubation of the homogenates facilitated the cleavage between the sialic acid in ESF and the basic groups of another substance. Whether such a process is operative *in vivo* during hypoxia can not be ascertained at this point. The lactic acidosis and changes in cellular redox potentials wrought by hypoxia, however, could affect hydrolysis of such bonds.

The chronic intermittent hypoxic schedule described in the present work involved acclimatization to hypoxia. This is a rather complex physiologic process with several unknown parameters. The participation of the j.g. cells in acclimatization is certainly suggested by the data in the altitude experiments. Whether the resultant increased secretory activities of the j.g. cells also caused an increase in plasma angiotensin, through their release of renin, is not known. Angiotensin could be involved in acclimatization through its direct cardiovascular effects and its aldosterone and adrenal cortex stimulating properties.[5,6]

It has been shown that renin is not erythropoietin[27] nor is the polypeptide, angiotensin, related to ESF. If the granular j.g. cells do elaborate erythropoietin this would be their second endocrine product. This is harmonious with the ultrastructural demonstration of the variety of granules seen in the j.g. cells.[28]

Summary

The renal granular juxtaglomerular cells have been shown to respond to hypoxia. Chronic intermittent exposures to simulated high altitudes did not involve a decrease in the size of the renal vascular bed. In spite of this, the j.g. cells showed increased secretory activities.

It was shown, furthermore, that the release of large amounts of erythropoietin could be correlated with a secretory discharge of granules from the j.g. cells. A method of extracting erythropoietin from renal cortices was devised and was useful in showing a correlation between the granularity of the j.g. cells and the amount of extractable renal erythropoietin.

The increased secretory activities of the j.g. cells are felt to play a role in acclimatization to high altitudes, firstly by increasing the circulating red cell mass through the liberation of erythropoietin and secondly by the release of the enzyme renin which causes an increase in the production of angiotensin. This in turn affects aldosterone production by the adrenal cortex.

REFERENCES

1. RUYTER, J. H. C. Ueber einen merkwürdigen Abschnitt der Vasa afferentitia in der Maüseniere. *Z. Zellforsch.*, 1925, **2**, 242–253.
2. GOORMAGHTIGH, N. Histological changes in the ischemic kidney; with special reference to the juxtaglomerular apparatus. *Am. J. Path.*, 1940, **16**, 409–416.
3. DEMOPOULOS, H.; KALEY, G., and ZWEIFACH, B. W. The Histologic distribution of renin in the kidneys of the rat and rabbit. *Am. J. Path.*, 1960, **37**, 443–455.
4. HARTROFT, P. M., and EDELMAN, R. Renal Juxtaglomerular Cells in Sodium Deficiency. Effect of Hypophysectomy. Localization of Renin by the Fluorescent Antibody Technique. In: Edema, Mechanisms and Management. Hahnemann Symposium on Edema. W. B. Saunders Co., Philadelphia, 1960, 63–68.
5. DAVIS, J. O.; AYERS, C. R., and CARPENTER, C. C. J. Renal origin of an aldosterone-stimulating hormone in dogs with thoracic caval constriction and in sodium-depleted dogs. *J. Clin. Invest.*, 1961, **40**, 1466–1472.
6. DAVIS, J. O. Importance of the renin-angiotensin system in the control of aldosterone secretion. In: Hormones and the Kidney. Academic Press, London and New York, 1963, 325–339.
7. DEMOPOULOS, H. B.; KALEY, G., and ZWEIFACH, B. W. Response of granular juxtaglomerular cells and tissue mast cells in various experimental states. *Circulation Res.*, 1961, **9**, 845–850.
8. TOBIAN, L. Interrelationship of electrolytes, juxtaglomerular cells and hypertension. *Physiol. Rev.*, 1960, **40**, 280–312.
9. PITCOCK, J. A., and HARTROFT, P. M. The juxtaglomerular cells in man and their relationship to the level of plasma sodium and the zona glomerulosa of the adrenal cortex. *Am. J. Path.*, 1958, **34**, 863–884.
10. YAZGAN, S. Investigations sur l'appareil juxtaglomerulaire. *Acta Anat.* (Basel), 1962, **48**, 368–388.
11. BARD, P. Medical Physiology. C. V. Mosby Co., St. Louis, 1961, 716–719.
12. SILVETTE, H. Some effects of low barometric pressures on kidney function in the white rat. *Am. J. Physiol.*, 1943, **140**, 374–386.
13. REISSMANN, K. R. Studies on the mechanism of erythropoietic stimulation in parabiotic rats during hypoxia. *Blood*, 1950, **5**, 372-380.
14. STOHLMAN, F., JR., and BRECHER, B. Humoral regulation of erythropoiesis V. relationship of plasma erythropoietin level to bone marrow activity. *Proc. Soc. Exper. Biol. & Med.*, 1959, **100**, 40–43.
15. LOWY, P. H.; KEIGHLEY, G., and BORSOOK, H. Inactivation of erythropoietin by neuraminidase and by mild substitution reactions. *Nature, London*, 1960, **185**, 102–103.
16. KURATOWSKA, Z.; LEWARTOWSKI, B., and MICHALAK, E. Studies on the production of erythropoietin by isolated perfused organs. *Blood*, 1961, **18**, 527–534.
17. SOKABE, H., and GROLLMAN, A. Localization of blood pressure regulating and erythropoietic functions in rat kidney. *Am. J. Physiol.*, 1962, **203**, 991–994.
18. TAKAKU, F.: HIRASHIMA, K., and NAKAO, K. Studies on the mechanism of erythropoietin production. I. Effect of unilateral constriction of the renal artery. *J. Lab. & Clin. Med.*, 1961, **59**, 815–820.
19. HIRASHIMA, K., and TAKAKU, F. Experimental studies on erythropoietin. II. The relationship between juxtaglomerular cells and erythropoietin. *Blood*, 1962, **20**, 1–8.

20. GORDON, A. S.; PILIERO, S. J.; MEDICI, P. T.; SIEGEL, C. D., and TANNENBAUM, M. Attempts to identify site of production of circulating 'Erythropoietin'. *Proc. Soc. Exper. Biol. & Med.*, 1956, **92**, 598–602.

21. RAMBACH, W. A.; ALT, H. L., and COOPER, J. A. D. Erythropoietic activity of tissue homogenates. *Proc. Soc. Exper. Biol. & Med.*, 1961, **108**, 793–796.

22. KEIGHLEY, G.; LOWY, P. H.; BORSOOK, H.; GOLDWASSER, E.; GORDON, A. S.; PRENTICE, T. C.; RAMBACH, W. A.; STOHLMAN, F., JR., and VAN DYKE, D. C. A cooperative assay of a sample with erythropoietic stimulating activity. *Blood*, 1960, **16**, 1424–1432.

23. HARTROFT, P. M., and HARTROFT, W. S. Studies on renal juxtaglomerular cells; I. Variations produced by sodium chloride and desoxycorticosterone acetate. *J. Exper. Med.*, 1953, **97**, 415–428.

24. HIGHMAN, B., and ALTLAND, P. D. Effect of altitude and cobalt polycythemia, hypoxia, and cortisone on susceptibility of rats to endocarditis. *Circulation Res.*, 1955, **3**, 351–356.

25. MILLER, G., and HARTROFT, P. M. Renal juxtaglomerular cells in acute dietary sodium deficiency. *Fed. Proc.*, 1961, **20**, 404.

26. GOLDFARB, B., and TOBIAN, L. The interrelationship of hypoxia, erythropoietin, and the renal juxtaglomerular cell. *Proc. Soc. Exper. Biol. & Med.*, 1962, **111**, 510–511.

27. KALEY, G., and DEMOPOULOS, H. B. Effect of erythropoietic stimuli on renin content and juxtaglomerular cells. *Fed. Proc.*, 1963, **22**, 664.

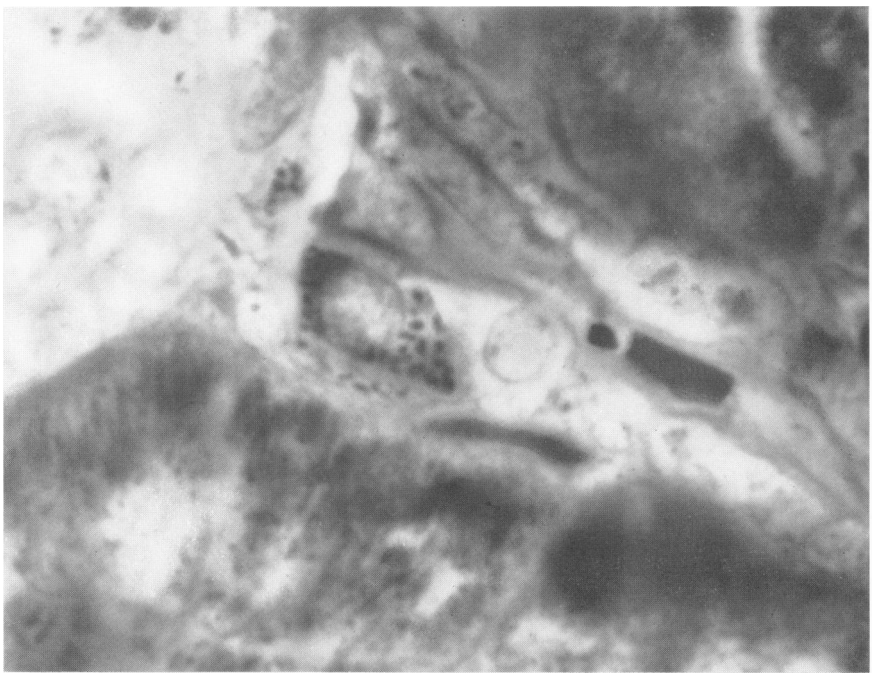
28. LATTA, H., and MAUNSBACH, A. B. The juxtaglomerular apparatus as studied electron microscopically. J. Ultrastruct. Res., 1962, **6**, 547–561.

[*Illustrations follow*]

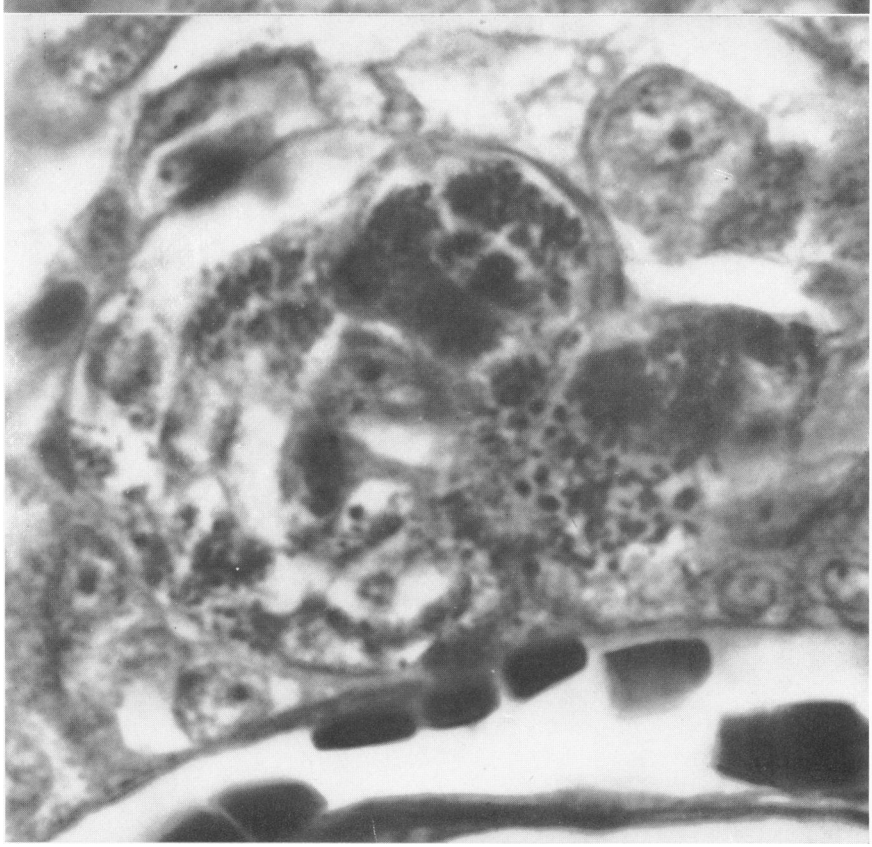
LEGENDS FOR FIGURES

FIG. 1. Afferent renal arteriole, normal control rat. Two granulated cells are evident in its wall. This number of cells and the degree of granulation is representative of normal rats. Bowie stain, × 1,200.

FIG. 2. Afferent arteriole, rat exposed intermittently to a simulated altitude of 25,000 feet for 5 days, allowed a weekend of rest, and sacrificed on Monday of the second week. Three to 4 cells in the arteriolar wall are heavily granulated. Approximately one third of the granular juxtaglomerular complexes encountered in such kidneys showed this degree of increased granularity. Bowie stain, × 1,200.



1



2