lung, spleen, and liver and of mouse embryo support the multiplication of the lactic dehydrogenase-elevating virus. Such cultures produced virus continuously until they had been subcultured 2-3 times. This corresponded to 20 weeks in the case of lung and spleen and to 2-3 weeks with cultures of embryo. Viral multiplication was not accompanied by cytologic alterations in the cells or by changes in their rate of synthesis of nucleic acids or protein. Infection did not cause detectable changes in either the production of LDH or in its release from cells.

1. Riley, V., Lilly, F., Huerto, E., Bardell, D., Science, 1960, v132, 545.
2. Adams, D. H., Rowson, K. E. K., Salaman, M. H., Brit. J. Cancer, 1961, v15, 860.
3. Mundy, J., Williams, P. C., Science, 1961, v134, 834.
4. Plagemann, P. G. W., Watanabe, M., Swim, H. E., Proc. Soc. Exp. Biol. and Med., 1962, v111, 749.
5. Notkins, A. L., Bacteriol. Rev., 1965, v29, 143.
6. Notkins, A. L., Greenfield, R. E., Marshal, D., Bane, L., J. Exp. Med., 1963, v117, 185.
7. Plagemann, P. G. W., Gregory, K. F., Swim, H. E., Chan, K. K. W., Can. J. Microbiol., 1963, v9, 75.
8. Notkins, A. L., Shochat, S. J., J. Exp. Med., 1963, v117, 735.
9. Plagemann, P. G. W., Swim, H. E., Proc. Soc. Exp. Biol. and Med., 1966, v121, 1142.
10. Earle W. R., Highhouse, F., J. Nat. Cancer Inst., 1954, v14, 841.
11. Swim, H. E., Parker, R. F., Am. J. Hyg., 1957, v66, 235.
12. Wroblewski, F., LaDue, J. S., Proc. Soc. Exp. Biol. and Med., 1955, v90, 210.
13. Plagemann, P. G. W., Swim, H. E., J. Bacteriol., 1966, submitted.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., J. Biol. Chem., 1951, v193, 265.
15. Sanford, K. K., Earle, W. R., Likely, G. D., J. Nat. Cancer Inst., 1948-49, v9, 229.
16. Gey, G. O., Coffman, W. D., Kubicek, M. T., Cancer Res., 1952, v12, 264.
17. Gross, L., Oncogenic Viruses, Pergamon Press, New York, 1961.
18. Yaffe, D., Cancer Res., 1962, v22, 573.
19. Evans, R., J. Gen. Microbiol., 1964, v37, vii.
20. Georgii, A., Lenz, I., Zobi, H., Proc. Soc. Exp. Biol. and Med., 1964, v117, 322.
21. Bailey, J. M., Clough, J., Stearman, M., ibid., 1964, v117, 350.
22. Mahy, B. W. J., Virology, 1964, v24, 481.
23. Notkins, A. L., Scheele, C., J. Nat. Cancer Inst., 1964, v33, 741.

Received November 29, 1965. P.S.E.B.M., 1966, v121.

Effect of Renin, Angiotensin II and Aldosterone on Erythropoiesis. (30992)

D. L. MANN, R. M. DONATI AND N. I. GALLAGHER
Department of Internal Medicine, St. Louis University School of Medicine and Radioisotope Laboratory, St. Louis Veterans Administration Hospital, St. Louis, Mo.

Although ten years have elapsed since the initial demonstration of the relationship of the kidney to erythropoietin(1), a specific renal site of erythropoietin production has not been found. Diminished juxtaglomerular cell granularity in bled anemic mice(2) focused attention on the juxtaglomerular apparatus. In contrast, further investigation(3-5) has shown hyperplasia of the juxtaglomerular apparatus or an increase in the granularity of the juxtaglomerular cells in experimental situations wherein erythropoietin production is augmented.

Increased juxtaglomerular granularity has also been noted when there is augmented production of renin, a substance known to be elaborated by the juxtaglomerular cells(6-8). The interrelationship of renin, angiotensin II, and aldosterone in regulation of arterial pressure and fluid balance has been established (6-8). Thus a possible explanation for the coincidence of increased juxtaglomerular granularity and augmented erythropoietic stimulating activity is that renin, angiotensin or aldosterone exert direct or indirect erythropoietic effects. Fisher and Crook(9) and

Piliego and Rossini(10) have reported that angiotensin II has erythropoietic activity. Bilsel and his associates(11) were unable to confirm these results. In the present study, angiotensin II, aldosterone and renin demonstrated no erythropoietic stimulating activity. Moreover, salt deprivation known to produce augmented renin, angiotensin and aldosterone elaboration(7-8) produced no alteration in red blood cell mass.

Methods. Female Sprague-Dawley rats weighing 180-200 g, previously fed a diet of Purina Laboratory Chow,* were starved for 96 hours and allowed water *ad libitum.* In one experiment, test material was injected I.P 24 and 48 hours after the initiation of starvation. In another experiment injections were given 48 and 72 hours after starvation was begun. The test materials given were angiotensin II,† 200 μg; renin,‡ 1500 μg; aldosterone,§ 2 μg; 0.9% saline or an extract of plasma from rabbits rendered anemic by phenylhydrazine administration(12). Eighteen hours prior to completion of starvation and sacrifice, 1 μc ^{59}Fe/1 μg ^{56}Fe citrate was administered *via* the tail vein. Eighteen hours later blood was obtained by cardiac puncture and the radioactivity of an aliquot determined in a well-type scintillation counter. Percent red blood cell ^{59}Fe incorporation was then determined using 5% total body weight as the estimated blood volume(12).

To determine the effects of increased endogenous production of renin, angiotensin, and aldosterone(6-8), sodium-deficient diets‖ and water were offered *ad libitum* to 20 female Sprague-Dawley rats weighing 180-200 g. A similar number of rats were given Purina Laboratory Chow and water. Hemoglobin and micro hematocrits were determined on each animal prior to initiation of the diet. After 3 weeks' maintenance on these diets, hemoglobin concentration, micro hematocrits and red blood cell mass were determined by ^{59}Fe tagged homologous cells(13).

Results. The results of the first experiments are presented in Table I. The % red blood cell ^{59}Fe incorporation was not increased over control values by administration of angiotensin II, renin or aldosterone, while the known erythropoietic stimulus of anemic rabbit plasma induced an approximate 6-fold increase. These values were subjected to statistical analysis by Student's "t" test; the only significant increase noted is the values obtained in the animals given anemic rabbit plasma.

Pertinent hematologic data resulting from the sodium deprivation is presented in Table II. No alteration in hemoglobin concentration, hematocrit, or red cell mass was observed in the animals on sodium free diets after a period of 3 weeks when compared to the rats on the liberal sodium diet.

Discussion. Attempts to localize the renal site of erythropoietin production have led to conflicting results. Fluorescent antibody techniques have localized erythropoietin antibody on glomerular tufts(14) suggesting this site of production whereas Osnes' observation suggested the cellular origin to be the juxtaglomerular apparatus. Hirashima and Takaku (3) induced increased granularity of the juxtaglomerular cell in rats made anemic by phenylhydrazine and bleeding, and decreased granularity by transfusion induced polycythemia. In addition, Kaley and Demopoulos (15) have reported increase in the granularity following cobalt administration or hypoxic exposure, stimuli known to augment erythropoiesis. Erythrocytosis, reticulocytosis, and increased level of erythropoietin have also resulted in animals following unilateral renal artery constriction with an increase in the granularity of the juxtaglomerular cells(16).

Experimental conditions which produce alterations in juxtaglomerular cell granularity and increased levels of erythropoietin involve hemodynamic changes as well as alterations in red cell mass. Since the juxtaglomerular apparatus responds to change in intravascular volume, this alteration in granularity may be unrelated to erythropoietin elaboration *per se.* Evidence for the production of erythropoietin by the juxtaglomerular cells is at best

* Iron content 275 p.p.m. Sodium content 0.9%.

† Procured as hypertensin, CIBA, Lot 262400A.

‡ Procured as hog renin from Dr. H. M. Perry, St. Louis, Mo.

§ Procured as aldosterone, CIBA, Lot E 6403.

‖ Sodium Deficient Test Diet, procured from Nutritional Biochemicals Corp., Cleveland, Ohio. Iron content 475 p.p.m. Sodium content—not measurable.

TABLE I. Percent 18 Hour Red Blood Cell ^{59}Fe Incorporation.

	Assay material injected 24 and 48 hr	Assay material injected 48 and 72 hr
Assay material saline	4.1 ± 1.5 (5)†	5.8 ± 2.3 (5)†
Angiotensin II, 200 μg	4.5 ± .4 (5) N.S.*	5.1 ± 2.4 (5) N.S.*
Renin, 1500 μg	2.7 ± .4 (5) N.S.*	6.4 ± 1.8 (5) N.S.*
Aldosterone, 2 μg	3.9 ± 1.5 (5) N.S.*	5.4 ± 2.0 (5) N.S.*
PHARP	32.2 ± .6 (5) .001*	30.4 ± .8 (5) .001*

* P value determined by Students "t" test.
† Number of animals in each group.

indirect and at present inconclusive. However, the possibility remains that the juxtaglomerular cells produce 2 distinct humoral substances.

Induction of increased granularity of the juxtaglomerular cells by hypoxia and simultaneous increases in erythropoietin without alteration in the renin-aldosterone axis would favor the concept of elaboration of two distinct substances by these cells. Goldfarb and Tobian(4,5) have been unable to confirm these observations in rats at a simulated altitude of 19,000 feet for 12 hours. Alternatively the renin-angiotensin-aldosterone system may have erythropoietic stimulating properties. The direct effect of these substances on erythropoiesis was tested. No erythropoietic effect was observed in any instance.

Acute sodium deprivation has been found to be a strong stimulus to renin, angiotensin and aldosterone production. Saline extracts of kidneys from rats fed low sodium diets have been reported to have increased erythropoietic activity(4,8). However, in these studies, no augmentation in the red blood cell mass was noted following 3 weeks on a sodium-deficient diet.

Summary. Erythrocyte radioiron incorporation following administration of renin, angiotensin II or aldosterone was not altered suggesting that these substances do not have erythropoietic stimulating properties. In addition, maintenance of rats on a sodium-deficient diet for a 3-week period in order to effect increased endogenous renin and aldosterone production produced no alteration in the red blood cell mass. The significance of these results in relation to the renal origin of erythropoietin is discussed.

TABLE II. Hematological Alteration on 3-Week Salt-Free Diet and Regular Diet.

	No. rats	Hct vol, % ± S.D.	Red cell mass, ml/100 g body wt
Regular diet			
Before	10	40.5 ± 1.4	
After	10	45.0 ± 1.6	2.12 ± 3.5
Na-free diet			
Before	10	40.5 ± 1.5	
After	10	45.5 ± 2.5	1.96 ± .28

1. Editorial, New Engl. J. Med., 1965, v273, 824.
2. Osnes, S., Brit. Med. J., 1965, v1, 1153.
3. Hirashima, K., Takaku, F., Blood, 1962, v20, 1.
4. Goldfarb, B., Tobian, L., Proc. Soc. Exp. Biol. and Med., 1962, v111, 510.
5. ———, ibid., 1963, v112, 65.
6. Slater, J. D. H., Barbour, B. H., Henderson, H. H., Casper, A. G. T., Bartter, F. C., J. Clin. Invest., 1963, v42, 1504.
7. Hartroft, P. M., Hartroft, W. S., J. Exp. Med., 1953, v97, 415.
8. Tobian, L., Thompson, J., Twedt, R., Janeek, J., J. Clin. Invest., 1958, v37, 660.
9. Fisher, J. W., Crook, J. J., Blood, 1962, v19, 557.
10. Piliego, N., Rossini, P., Rass. Fisiop. Cl. Ter., 1963, v35, 687.
11. Bilsel, V. C., Wood, J. E., Lange, R. D., Proc. Soc. Exp. Biol. and Med., 1963, v114, 475.
12. Gallagher, N. I., Hagan, D. Q., McCarthy, J. M., Lange, R. D., ibid., 1961, v106, 127.
13. Gibson, J. G., II, Weiss, S., Evans, R. D., Peacock, W. C., Irvine, J. W., Jr., Good, W. M., Kip, A. F., J. Clin. Invest., 1946, v106, 616.
14. Fisher, J. W., Taylor, G., Porteous, D. D., Proc. Am. Soc. Hemat., Nov. 15, 1964.
15. Kaley, G., Demopoulos, H. B., Fed. Proc., 1963, v22, 664.
16. Mitus, W. J., Toyama, K., Arch. Path., 1964, v78, 658.

Received November 29, 1965. P.S.E.B.M., 1966, v121.