Erythrocytosis in Spontaneously Hypertensive Rats

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A B S T R A C T During the study of an inbred strain of Wistar rats which spontaneously develop hypertension when they reach a weight of approximately 150 g, it was found that these animals also develop an erythrocytosis. A significant increase in red cell count was observed in spontaneously hypertensive (SH) rats (8–11 × 10^6 RBC/mm^3) when compared with normotensive rats (6–7 × 10^6 RBC/mm^3) of the same strain. This increase in red cell count paralleled the increase in body weight and the rise in blood pressure.

Since the plasma volume, as measured with labeled albumin was normal, there was an absolute increase in red cells. The hematocrit and hemoglobin content of the blood measured in SH rats were only slightly greater than those found in normotensive rats. However, the mean cell volume (MCV) of the red cells in the SH rats was 45–47 μ^3 as compared with 51–53 μ^3 in normotensive rats.

A fourfold increase in 24 hr ^{59}Fe incorporation into the red cells was found in the SH rats when compared with normotensive controls. The bone marrow of the SH rats showed erythroid hyperplasia. When the SH rats were treated with α-methyldopa (Aldomet 200 mg/kg daily, i.p.) the red cell count fell in parallel with the drop in blood pressure. No change in red cell count or blood pressure was observed in normotensive rats treated in the same manner. The erythropoietin titer was high in SH rats, and was undetectable in normotensive rats. These observations suggest a direct relationship between the hypertension and the erythrocytosis mediated by erythropoietin; both are genetically controlled.

INTRODUCTION

Okamoto and Aoki (1) reported that they had bred a strain of Wistar rats which spontaneously develop hypertension. The blood pressure in these rats remains constant and normal until they reach a weight of approximately 150 g from which time the blood pressure gradually rises in direct proportion to their weight. During the investigation of the cause of this hypertension, we found that the red cell count rises concommitantly and in direct proportion to the blood pressure. This paper reports our findings concerning the nature and cause of this erythrocytosis and its relationship to the hypertension.

METHODS

The spontaneously hypertensive Wistar rats (SH)[1] used in this study were either bred at the Cleveland Clinic Foundation or obtained from Purina Laboratory.[2] Normal controls were either Sprague Dawley rats[3] or rats from a normotensive Wistar strain.[4] All rats were fed on commercial rat chow diet and tap water ad lib. Renal hypertensive rats were prepared by applying a silver clip to the left renal artery under ether anesthesia (2).

Blood pressure was measured at the same time of the day by the same person, using a tail cuff as described by Friedman and Freed (3). Blood was collected from the orbital cavity in conscious rats, from the tail artery under ether anesthesia or from the aorta when the animal was sacrificed, using sodium ethylenediaminetetraacetate (EDTA) as anticoagulant.

Blood counts and red cell indices were determined on a model S Coulter particle counter and platelets were counted on a model F Coulter particle counter.[5] Blood and bone marrow films were stained with Wright's stain, and reticulocytes were counted on blood films stained with brilliant cresyl blue.

Incorporation of radioactive iron into circulating red cells was reported as the percentage of a dose of 2 μCi ^{59}Fe^{++} citrate in 0.1 ml saline reappearing in the circulation 23 hr

[1] *Abbreviations used in this paper:* IRP, international reference preparation; SH, spontaneously hypertensive.
[2] Purina Laboratory Animals, Vincentown, N. J.
[3] Carworth Division, Becton, Dickinson & Co., New York.
[4] Hilltop Lab Animals, Inc., Scottdale, Pa.
[5] Coulter Electronics, Inc., Hialeah, Fla.

TABLE I

Hematological Characteristics of Normotensive, Spontaneously Hypertensive, and Renal Hypertensive Rats

	Normal rats	Spontaneously hypertensive rats				Renal hypertensive rats
Weight, g	200–300	130–150	180–200	200–250	250–300	200–300
Blood pressure, *mm Hg*	110–120	110–130	180	200	200	210
Number	25	10	25	25	25	8
RBC × 10⁶/mm³	6.8	6.4	8.1	8.7	10.0	6.8
	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)	(0.1)
WBC × 10³/mm³	9.2	8.4	9.3	8.4	10.6	10.5
	(0.3)	(0.5)	(0.3)	(0.4)	(0.5)	(0.6)
Hemoglobin, g/*100 ml*	14.7	13.5	14.8	15.7	16.6	13.3
	(0.2)	(0.2)	(0.1)	(0.2)	(0.1)	(0.7)
Hematocrit, %	37.6	36.5	37.6	40.8	44.1	39.8
	(0.6)	(0.5)	(0.1)	(0.3)	(0.4)	(0.5)
MCV, μ³	54.2	55.5	47.0	45.8	45.4	53.5
	(0.6)	(0.6)	(0.2)	(0.1)	(0.1)	(0.6)

Mean, with standard error of the mean in parenthesis.

after intravenous injection. The following formula was used:

Per cent incorporation ^{59}Fe =

$$\frac{\text{Radioactive counts/ml whole blood at } 23 \text{ hr} \times \text{blood volume (ml)}}{\text{Total radioactive counts injected}}$$

Plasma volume was measured as the albumin-^{131}I distribution space 20 min after the injection of 5 μCi tagged albumin. Autologous red cells collected in acid-citrate-dextrose and labeled with ^{51}Cr sodium chromate were used to measure red cell survival. The iliac artery was canulated under sodium amytal anesthesia (75 mg/kg i.p.); 1.5 ml blood was withdrawn, labeled, and then reinjected through the cannula. 20 min later 0.3 ml blood was collected and the cannula removed. This specimen was used to calculate the 100% level for red cell survival. Rats were bled at weekly intervals and radioactivity measured. Plasma iron was measured by atomic absorption.

Erythropoietin was measured by Dr. James Fisher (Department of Pharmacology, Tulane University), by injecting 1 ml of serum (0.5 ml daily for 2 days) into exhypoxic polycythemic mice. The results were expressed as per cent ^{59}Fe incorporation into RBC and converted into IRP (international Reference Preparation) units per milliliter from a log dose-response curve (4). α-Methyldeoxyphenylalanine (α-methyldopa) was given in a single dose of 200 mg/kg i.p. daily.

RESULTS

The hematological characteristics of normotensive, spontaneously hypertensive, and renal hypertensive rats are
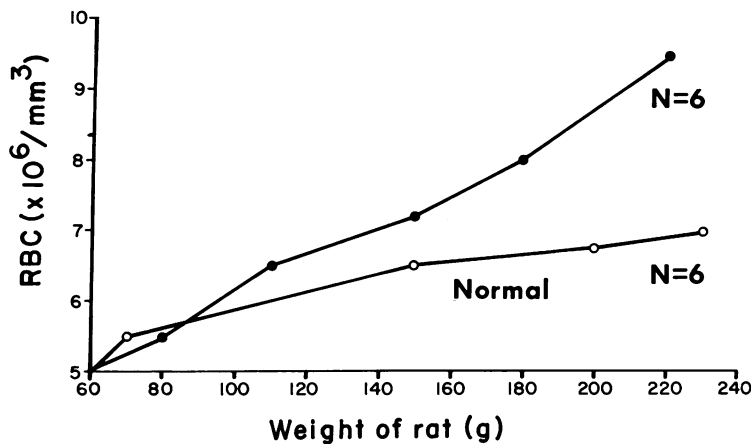

FIGURE 1 Red cell counts in six normal (○) and six SH (●) rats studied for 8 wk as their weight increased from 70 to 230 g.
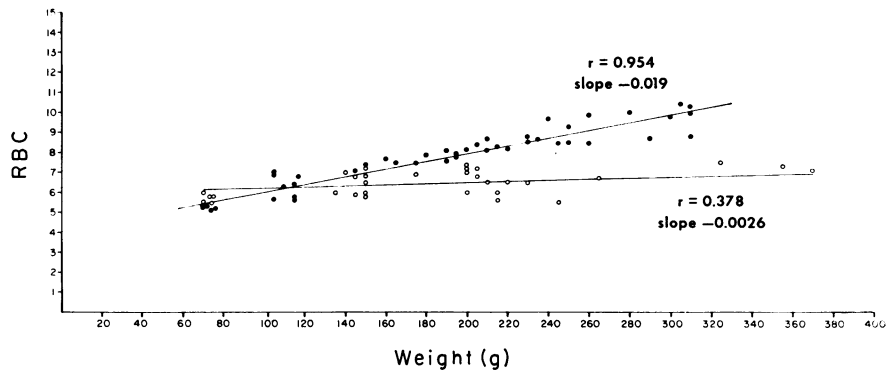
FIGURE 2 Red cell counts of 150 SH rats and 75 normal rats plotted against their weight in grams. Regression lines indicate a direct relationship in SH rats ($r = 0.95$, $P < 0.0001$) and a lack of relationship ($r = 0.38$, $P < 0.4$) in normal rats. ●, SH rats; ○, normal rats.

presented in Table I. These characteristics were studied in rats of both sexes, but since no difference in red cell counts was observed mixed group data are reported. The results of the blood counts show that the red cell count in the SH rats increases as the weight and blood pressure increased. The red cells become smaller in size and this is reflected in a relatively smaller increase in blood hemoglobin content and hematocrit. The mean plasma volume of 15 normal rats was 39.08 ml/kg (SE 0.46) and that of 15 SH rats was 39.08 ml/kg (SE 0.88). A comparison between the body weight and red cell

count shows a direct relationship based on a regression line calculated for the data shown in Fig. 2 (r = 0.95, P < 0.0001). The red cell count and body weight were measured at weekly intervals in six normal rats and six SH rats and the results (Fig. 1) were similar to those found in the larger groups (Fig. 2) which were not followed individually. The red cell counts also showed a linear relationship to the blood pressure ($r = 0.95$, $P < 0.0001$ Fig. 3). The mean quantity of radioactive iron (^{59}Fe) incorporated into red cells 23 hr after injection was 8.8% in five normotensive rats with a mean red
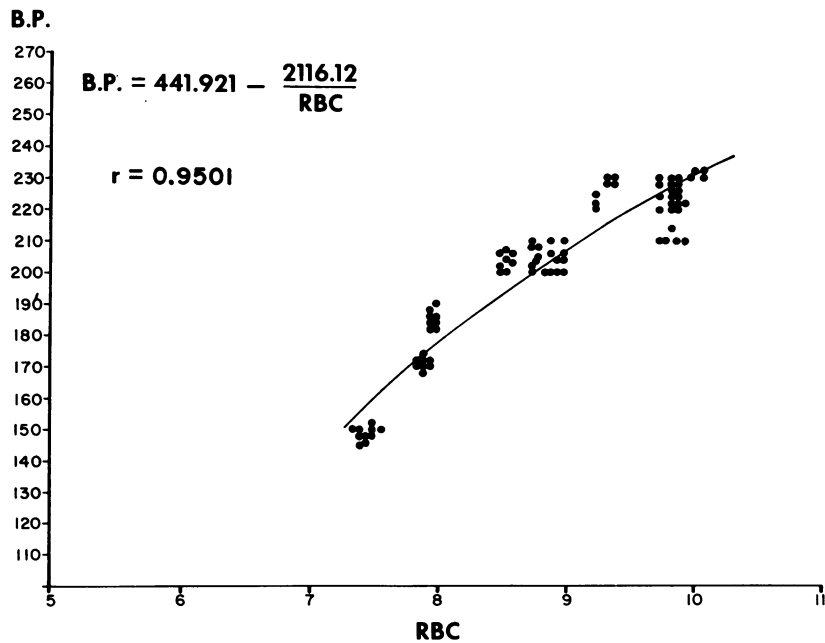

FIGURE 3 Blood pressure of 75 SH rats plotted against their red cell counts. Regression line indicates a direct but not linear relationship ($r = 0.95$, $P < 0.0001$); the regression formula is shown.

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TABLE II

Effect of α-Methyldopa (Aldomet) on Spontaneously Hypertensive and Normotensive Rats

	Normotensive			Spontaneously hypertensive		
	Before*	During‡	After§	Before*	During‡	After§
Blood pressure, mm Hg	110 ±10	100 ±10	100 ±5	200 ±10	145 ±15	200 ±10
RBC × 10⁶/mm³ Erythrocyte counts, (×10⁶/mm³)	6.3 ±0.2	6.5 ±0.3	6.5 ±0.3	10.3 ±0.1	6.18 ±0.1	8.9 ±0.2
Plasma volume, ml	9.3 ±0.2	8.5 ±0.4		9.5 ±0.5	7.9 ±0.6	

Results expressed as mean ±SEM of eight rats.
* Control data before drug treatment.
‡ Data obtained after 18 days of drug treatment.
§ Data obtained 14 days after discontinuation of drug.

cell count of 6.5 × 10⁶/mm³ and blood pressure of 100 mm Hg, and 41% (se 2.16) in five SH rats with a mean red cell count of 9.8 × 10⁶/mm³ and blood pressure of 200 mm Hg. Red cell survival (t½) was 9–16 days in five normal and nine to greater than 16 days in five SH rats. The reticulocyte count ranged from 140 to 210 × 10³/mm³ in normal rats and ranged from 140 to 270 × 10³/mm³ in SH rats. The bone marrow of three SH rats showed a marked erythroid hyperplasia when compared with the marrow of three normotensive rats. The marrow of one normotensive and one hypertensive rat were stained for iron; iron was present in both.

The plasma iron in two normal rats was 256 and 270 μg/100 ml and in two hypertensive rats was 224 and 230 μg/100 ml.

Treatment with α-methyldopa reduced the blood pressure and the red cell count in SH rats but affected neither parameter in normotensive rats (Table II). The reticulocyte count fell to a range of 7–7.5 × 10³/mm³ in the SH rats. Discontinuation of the drug treatment

led to a return of both hypertension and erythrocytosis in the SH rats. A typical response to a α-methyldopa treatment is shown in Fig. 4. A similar effect was seen when guanethidine and hexamethonium were used to reduce the blood pressure. The greatest decrease in red cell count was seen in those SH rats that had the greatest fall in blood pressure. Serum erythropoietin titers are listed in Table III. The younger rats (150–160 g) have the highest titer 0.27 IRP U/ml. The titers in older rats were 0.15 and 0.17 IRP U/ml in 180-g rats and 250-g rats respectively. The erythropoietin titer was not detectable in normal rats.

DISCUSSION

The results of these studies show that the strain of Wistar rats which spontaneously develop hypertension also develop an erythrocytosis. Platelet and white cell production are not affected. Plasma volume measurements indicate that the erythrocytosis is absolute. The erythroid hyperplasia in the bone marrow and the four-
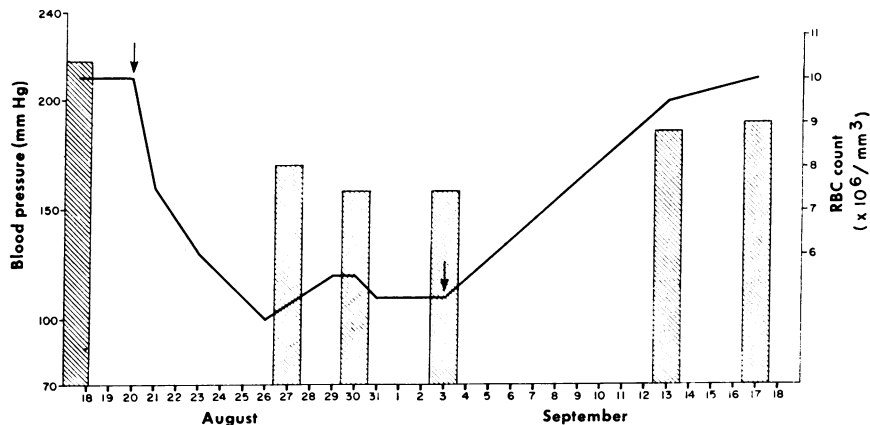

FIGURE 4 Typical changes of blood pressure and red cell count in a SH rat in response to α-methyldopa treatment. Bars represent red cell count and line represents blood pressure. The first arrow indicates the beginning of treatment with Aldomet (200 mg/kg). The second arrow indicates the withdrawal of drug.

TABLE III

Erythropoietin Titer in Serum of Spontaneously Hypertensive and Normotensive Rats

Assay material	No. of assay mice	^{59}Fe	ESF U/ml (IRP)
		%	
Saline	6	1.7	none
0.2 U IRP	5	8.11	
1.0 U IRP	5	27.14	
Pooled serum			
SH rats (150–160 g) n = 4	5	11.80	0.27
SH rats (180 g) n = 3	5	4.08	0.15
SH rats (250–270 g) n = 3	5	6.14	0.17
Normotensive rats n = 4	5	0.75	none

Results expressed as per cent ^{59}Fe incorporation and IRP* units per milliliter.

fold increase in iron turnover are further evidence of increased red cell production. The red cells that are produced during this phase have a significantly smaller MCV than the cells produced before the development of erythrocytosis. As a result, the blood hemoglobin content and hematocrit increase at a slower rate and this may in part account for the fact that the erythrocytosis has not been previously reported. The survival of these small red cells is normal. The reduction in size does not appear to be due to iron lack.

Erythropoietin is apparently the immediate stimulus to the increased red cell production. Plasma levels of the hormone are highest at the onset of the erythrocytosis but remain very high even when the red cell count is $10 \times 10^6/mm^3$. The direct correlation between the blood pressure and the erythrocytosis suggests a causal relationship. Not only does the red cell count increase as the blood pressure rises, but also the red cell count decreases when the blood pressure is lowered by various antihypertensive drugs. Furthermore, when the drug treatment is discontinued, the blood pressure and the red cell count increase again. We have no evidence as to the source of erythropoietin in these SH rats. However, the kidney is a major source of erythropoietin (5). Fisher and Langston (6) suggest that renal hypoxia may be the stimulus to its production. Analysis of blood gases showed no change in oxygen tension in the arterial blood as the red cell count increased. One could postulate that hypertension reduces the renal blood flow or alters its intrarenal distribution in such a way that oxygen supply to the erythropoietin-producing cells is reduced.

Aoki (7) has shown that adrenalectomy reduces the blood pressure in the SH rats. We have studied four adrenalectomized SH rats and the red cell count decreased from a mean of 10–$6.5 \times 10^6/mm^3$ in 14 days and the blood pressure decreased from 200 to 150 mm Hg. Although plasma volumes were not measured, these preliminary studies suggest that the adrenal gland may be involved in the production of hypertension and secondarily in the production of erythropoiesis. In this connection Waldmann and Bradley (8) reported the association of polycythemia and hypertension in a patient with pheochromocytoma and demonstrated the presence of an erythropoiesis-stimulating substance in chromaffin tumors.

Whatever the ultimate cause of the erythrocytosis and the hypertension is proven to be, the SH rats provide a readily available model for the study of spontaneous erythrocytosis due to increased erythropoietin production.

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