Similarties of Essential and Spontaneous Hypertension
Volume and Number of Blood Cells

Giacomo Bruschi, Marilena Minari, Maria E. Bruschi, Luisa Tacinelli, Barbara Milani, Angelo Cavatorta, and Alberico Borghetti

SUMMARY Spontaneously hypertensive rats have long been used as an animal counterpart of human essential hypertension. The validation of this strain as a model rests mainly on the "clinical" similarity of the two syndromes, but it has scarcely been founded on numerical comparison of measurable parameters. We investigated three hematological indexes previously recognized to be altered in spontaneously hypertensive rats: the single-cell volume of erythrocytes, the single-cell volume of platelets, and the erythrocyte number. Erythrocyte volume was lower by 7%, platelet volume was higher by 12%, and erythrocyte count was higher by 22% in spontaneously hypertensive rats in comparison with Wistar-Kyoto controls. More unexpectedly, it was found that erythrocyte volume is lower by 2%, platelet volume is higher by 3%, and erythrocyte number is higher by 6% in essential hypertensive subjects when compared with normotensive healthy subjects. These results, combined with previously reported blood cell alterations in subjects and rats, reinforce the evidence of a biological similarity between essential and spontaneous hypertension.

KEY WORDS • essential hypertension • spontaneously hypertensive rats • blood cells • cell volume • erythrocyte volume • erythrocyte count • platelet volume • platelet count

The usefulness of adequate animal models of essential hypertension requires no special emphasis. Spontaneously hypertensive rats (SHR) of the Okamoto-Aoki strain are the most widely accepted such model at the present time. Since their introduction, considerable evidence has been accumulated that essential and spontaneous hypertension share many common clinical features (e.g., genetic transmission, natural history, pathology, complications); however, this is a rather vague and unsatisfactory assessment of the possibility that the two syndromes represent the same disease in different species. Actually, this assumption has been challenged. Moreover, the proliferation of different strains with apparently different characteristics makes the presence of genetic hypertension with its clinical manifestations an uncertain criterion for considering a spontaneously hypertensive strain as a model. A comparison of precise, quantitative biological indexes would be more suitable to prove or disprove the similarity of essential and spontaneous hypertension.

In the search for such indexes, an example is at hand. Okamoto-Aoki SHR have long been known to possess curious hematological features, such as smaller and more numerous erythrocytes and larger platelets. Apparently, no such alterations have been reported in essential hypertensive patients. However, we recently realized that some defects in blood cells were expressed to a much greater degree in SHR than in essential hypertensive patients; in the latter, a large number of subjects is required to demonstrate them. Therefore, the design of this study was to examine the size and number of platelets and erythrocytes in large populations of patients and controls, as well as in SHR and control Wistar-Kyoto rats (WKY) at different ages. To gain insight into the relationship between blood cell defects and blood pressure, WKY were studied in which secondary hypertension was induced with deoxycorticosterone acetate (DOCA)–salt treatment. Humans with hypertension secondary to glomerulonephritis were also investigated.
Materials and Methods

Group Sampling
Spontaneously Hypertensive and Normotensive Wistar-Kyoto Rats

Pedigreed SHR (Okamoto-Aoki strain) derived from the National Institutes of Health colony were originally bought from the Centre de Selection et d’Elevage d’Animaux de Laboratoire (Centre National de la Recherche Scientifique, Orleans, France) and successively inbred in our laboratory for nine generations. Blood pressure, usually measured by tail plethysmography, was observed to follow a progressive, age-dependent course plateauling at about 4 months (Figure 1). Rats used in this study received standard chow laboratory diet and tap water ad libitum.

Deoxycorticosterone Acetate–Salt Hypertensive Rats

The DOCA–salt hypertension without unilateral nephrectomy was induced in 15 female 4-month-old WKY by the method of Kwan and Grover.7 This schedule implies more frequent doses of steroid than those given in usual protocols involving nephrectomy, followed by a washout period. After 1 week, 56% of the rats (10 of 18) were hypertensive (systolic blood pressure [SBP] > 160 mm Hg), and this percentage rose at a progressively slower rate until, after 5 weeks of treatment, 83% of the rats (15 of 18) were hypertensive. The administration of DOCA was stopped while the animals continued to drink 0.9% saline. After 20 days, the blood pressure was unchanged or slightly increased; the hypertensive rats were killed and studied; hence, these animals were closer to a post-DOCA model8 than to the classic pattern of mineralocorticoid-induced hypertension. Fifteen of their untreated sisters served as controls. Female rats used throughout this study were separated from male rats at weaning.

Essential Hypertensive Subjects and Normotensive Controls

The human study was begun in March 1984. Normotension was defined as SBP of 140 mm Hg or less and diastolic blood pressure (DBP) of 90 mm Hg or less. Hypertension was defined as SBP of more than 150 mm Hg or DBP of more than 95 mm Hg (i.e., subjects with borderline or labile hypertension were excluded). Individual blood pressure values in each subject were the mean of at least three determinations. The following four groups were studied.

Group 1 consisted of essential hypertensive patients admitted to the University Hospital of Parma (Istituto di Clinica Medica e Nefrologia and Istituto di Semiotica Medica) between July 1979 and October 1985. The diagnosis was established according to rules described elsewhere.1–2 Patients selected for this study fulfilled the following criteria: 1) they were untreated (i.e., either had never received any medication or had discontinued it at least 10 days before admission); 2) they were younger than 60 years of age; 3) they had normal renal function (i.e., routinely measured creatinine clearance greater than 70 ml/min or serum creatinine lower than 1.6 mg/ml, or both). Thus, these were relatively young patients, free from severe complications, whose rationale for admission usually was the search for possible secondary causes of their high blood pressure. This group contained 170 men and 121 women.

Group 2 consisted of normotensive subjects hospitalized between July 1979 and October 1985 for health
checkups (28%) or minor medical problems. The latter included subjective complaints (e.g., anxiety, nervousness, dizziness; 32%), mild benign orthostatic proteinuria (16%), sporadic microscopic hematuria (13%), and other problems (11%). The stay in the hospital proved that these were healthy subjects on the basis of history and physical, laboratory, and roentgenographic examinations. This group met the three conditions outlined above for the hypertensive subjects and contained 103 men and 91 women.

Groups 1 and 2 were geographically and racially homogeneous (i.e., they were whites of northern Italian origin, and about 90% of them lived within a 50-mile radius of Parma). Groups 1 and 2 received the same hospital diet. The percentage of hypertensive (50%) and normotensive (47%) smokers was similar. Other systematic environmental differences were not apparent. Seventeen percent of normotensive and 58% of hypertensive subjects were aware of having one first-degree relative with high blood pressure.

Group 3 consisted of essential hypertensive subjects with a previously established diagnosis who were customarily seen as outpatients. This group contained 51 men and 38 women.

Group 4 was composed of nonhospitalized, normotensive, healthy workers from among the university personnel who underwent medical examination and routine hematological tests at the time of some professional appointment. This group contained 149 men and 53 women.

Groups 3 and 4 were studied from March 1984 to October 1985. The three criteria defined for Group 1 were also applied to these groups.

Erythrocyte measurements in the four groups were performed by the same personnel and with the same equipment (the central laboratory of the university hospital). Platelet studies were performed by the authors only in subjects from Groups 3 and 4, who volunteered to participate after being informed about the purpose and method of this study.

Glomerulonephritis Patients

Glomerulonephritis (GN) patients hospitalized between 1979 and 1985 had a clinical diagnosis confirmed by histological examination of their kidney biopsy specimens. Patients selected for this study met the following criteria: 1) no treatment (directed against either their renal disease or hypertension) and 2) creatinine clearance greater than 70 ml/min and serum creatinine level less than 1.6 mg/ml; patients with GN associated with systemic disease (e.g., lupus erythematosus) were excluded.

Hypertensive and normotensive GN patients were homogeneous with respect to age, date of hospitalization, renal function, and histological diagnosis. The respective percentages of each type of GN in the hypertensive and normotensive GN groups are as follows:

1. Minimal changes: 4% and 2%
2. Focal sclerotic: 2% and 1%
3. Membranous: 16% and 18%
4. Proliferative: 15% and 8%
5. Membranoproliferative: 15% and 7%
6. Mesangio proliferative: 48% and 53%

According to these standards, some of the hypertensive GN patients could have essential hypertension independent of their kidney disease. Nevertheless, they should represent a small fraction of the hypertensive GN population, as 1) the prevalence of hypertension in the GN population was 40% (i.e., 5 times higher than the prevalence of essential hypertension in a general population of the same age composition; 150/95 mm Hg basis)9 and 2) the familial occurrence of hypertension was similar in the hypertensive and normotensive GN patients (data not shown).

Laboratory Procedures

Erythrocytes

In all six human groups, red blood cell mean corpuscular volume (RBCV), red blood cell number (RBCN), hematocrit, and hemoglobin were measured by the hospital laboratory with a Coulter counter (model S; Coulter Electronics, Luton, Bedfordshire, England). Blood was withdrawn from fasting recumbent subjects between 0730 and 0900, with ethylenediaminetetraacetic acid used as an anticoagulant, and analyzed at a 1:50,000 dilution according to standard procedures used for routine hematological tests. The instrument was calibrated daily with standard erythrocyte suspensions supplied by the manufacturer (4C; Coulter Electronics).

In rats, heparinized blood was withdrawn from the femoral artery with the animals under light ether anesthesia. The blood was diluted 1:50,000 with Isoton (Coulter Electronics) and immediately counted in a Coulter (Model ZM) counter at room temperature. The instrument was automatically corrected for coincidence counting. A volume histogram was established by repeated counting of 50 μl (the suspension volume pulled each time through the counting aperture) at successive 5-fl intervals from 5 fl up to 120 fl. Each counting time was about 5 seconds, and the total counting time was about 3 minutes. The mean cell volume of erythrocytes suspended in Isoton is stable within 0.3% at least 10 minutes. The mean cell volume and the total cell number were calculated from volume distribution histograms with the aid of microcomputer programs. Calibration was performed with 4C human erythrocyte suspensions.

Platelets

Platelet measurement procedures were similar in rats and humans. Blood was withdrawn from antecubital veins (humans) or femoral arteries (rats) into syringes containing (vol/vol) 1:10 (humans) or 1:6 (rats) acid-citrate-dextrose anticoagulant. Then 200 μl (humans) or 100 μl (rats) of blood was layered on a prefilled (0.45-μm pores) mixture of Ficoll-metrizoate-Isoton as described by Archer et al.10 The specific gravity of this cushion was 1.057 (humans) or 1.065
(rats), and its osmolarity and pH were 320 mosm/L and 7.4, respectively. The height of the cushion was 1.5 cm. The tubes were centrifuged at 150 g, 32°C, for 10 minutes (humans) or 5 minutes (rats). The principle of this method is that, by choosing an appropriate density and height of the gradient column and appropriate centrifugal force and time, the separation of platelets, which are smaller and lighter than other blood cells, can be optimized to obtain complete recovery. The accuracy of the method was judged by parallel countings on whole blood treated with 1% filtered ammonium oxalate under phase microscopy. Platelets, along with some lymphocytes, are trapped in the supernatant. This lymphocyte fraction can be readily discriminated as their size does not overlap with the volume distribution of platelets. We preferred this method of platelet isolation to standard sedimentation or centrifugation because the latter underestimates both platelet number and volume: the denser and larger platelets are lost at the top of, or within, the sedimented red and white blood cells.

Platelets in the supernatant were diluted 1:2000 in Isoton at 32°C, and a volume histogram was established as described for the erythrocytes, with 1-fl volume windows from 1 to 20 fl (rats) or 25 fl (humans). The total counting time was about 4 minutes. Platelet volume is stable in Isoton for at least 10 minutes. The mean cell volume was calculated from the histograms as previously described with microcomputer programs. The instrument was calibrated with 4C; this was preferred to latex particles as the latter underestimate the absolute cellular volume because of differences in electric impedance between plastic beads and living cells.

Platelets isolated on polysaccharide gradients have been shown to be morphologically and functionally intact; however, their volume invariance has not been thoroughly assessed. In the preliminary stage of this work, it was shown that 1) the volume of platelets isolated on gradients is identical to that of platelets fixed immediately at venipuncture and 2) platelets from platelet-rich plasma had the same volume whether they were diluted and counted immediately or after gradient centrifugation.

Results

Rats

The SHR differed significantly from WKY in having a smaller RBCV, a higher RBCN, and a greater platelet corpuscular volume (Table 1; see Figure 1). The platelet count was similar in the two strains. Typically, these changes occur progressively with age; very young SHR showed a tendency to exhibit the reverse changes (although differences were not significant). Hematological changes were slightly less pronounced in female than in male SHR (see Figure 1).

In DOCA-salt hypertensive rats, the RBCN and platelet corpuscular volume were not different from those of their genetically homogeneous, normotensive, untreated controls (see Table 1).

Humans

Male and female essential hypertensive patients differed from their respective normotensive control groups in having an increase in RBCN (7% in men and 4.4% in women), a decrease in RBCV (2.2% in men and 1.7% in women), and an increase in platelet corpuscular volume (3.9% in men and 3.4% in women; Tables 2 and 3; Figure 2). These changes were statistically significant. As in SHR, the absolute platelet count (per mm^3 of blood) was not altered in essential hypertensive patients.

Correlations between hematological variables and either SBP or DBP in hypertensive patients were characterized by the following coefficients: SBP/RBCN, 0.27; SBP/RBCV, -0.23; SBP/platelet corpuscular volume, 0.31; DBP/RBCN, 0.22; DBP/RBCV, -0.21; DBP platelet corpuscular volume, 0.26.

Hypertensive GN patients with normal glomerular

<table>
<thead>
<tr>
<th>Table 1. Blood Cell Indexes in Adult Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Male SHR</td>
</tr>
<tr>
<td>Male WKY</td>
</tr>
<tr>
<td>Female SHR</td>
</tr>
<tr>
<td>Female WKY</td>
</tr>
<tr>
<td>Female DOCA-salt hypertensive rats</td>
</tr>
<tr>
<td>Female DOCA-salt untreated controls</td>
</tr>
</tbody>
</table>

*Values are means ± SEM, except for systolic blood pressure, which shows mean values with extreme values in parentheses to the right. Figures in parentheses below the main numbers denote age in weeks and number of animals.  
*p < 0.0001, †p < 0.001, compared with values in their respective control groups.
Table 2. Red Blood Cell Indexes in Hypertensive and Normotensive Humans

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Age (yr)</th>
<th>Blood pressure (mm Hg)</th>
<th>Red blood cell number (10^6/\text{mm}^3)</th>
<th>Volume (fl)</th>
<th>Hematocrit (%)</th>
<th>Hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential hypertensive men</td>
<td>1</td>
<td>170</td>
<td>43(19–60)</td>
<td>178(111)</td>
<td>5.23 ± 0.04</td>
<td>87.8 ± 0.4</td>
<td>45.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>51</td>
<td>45(28–61)</td>
<td>171(105)</td>
<td>5.16 ± 0.06</td>
<td>87.6 ± 0.6</td>
<td>45.3 ± 0.6</td>
</tr>
<tr>
<td>Normotensive control men</td>
<td>2</td>
<td>103</td>
<td>45(19–60)</td>
<td>126(83)</td>
<td>4.84 ± 0.04</td>
<td>89.8 ± 0.5</td>
<td>43.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>149</td>
<td>39(24–59)</td>
<td>123(83)</td>
<td>4.90 ± 0.04</td>
<td>89.4 ± 0.4</td>
<td>43.9 ± 0.3</td>
</tr>
</tbody>
</table>

\( p \) Value

1 vs 2 <0.0001 <0.005 <0.0005 <0.0005
3 vs 4 <0.001 <0.02 <0.05 <0.001

Essential hypertensive women

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yr)</th>
<th>Blood pressure (mm Hg)</th>
<th>Red blood cell number (10^6/\text{mm}^3)</th>
<th>Volume (fl)</th>
<th>Hematocrit (%)</th>
<th>Hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>121</td>
<td>49(25–60)</td>
<td>172(107)</td>
<td>4.67 ± 0.04</td>
<td>87.5 ± 0.5</td>
<td>40.9 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>46(27–58)</td>
<td>167(103)</td>
<td>4.75 ± 0.06</td>
<td>87.4 ± 0.6</td>
<td>41.5 ± 0.6</td>
</tr>
</tbody>
</table>

\( p \) Value

1 vs 2 <0.0001 <0.05 <0.05 <0.01
3 vs 4 <0.01 <0.05 NS <0.05

Essential hypertensive women

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yr)</th>
<th>Blood pressure (mm Hg)</th>
<th>Red blood cell number (10^6/\text{mm}^3)</th>
<th>Volume (fl)</th>
<th>Hematocrit (%)</th>
<th>Hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>91</td>
<td>46(23–59)</td>
<td>119(80)</td>
<td>4.47 ± 0.04</td>
<td>89.0 ± 0.5</td>
<td>39.8 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>36(23–56)</td>
<td>120(81)</td>
<td>4.53 ± 0.05</td>
<td>88.9 ± 0.5</td>
<td>40.3 ± 0.4</td>
</tr>
</tbody>
</table>

\( p \) Value

1 vs 2 <0.001 <0.05 <0.05 <0.01
3 vs 4 <0.01 <0.05 NS <0.05

Glomerulonephritis hypertensive men

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yr)</th>
<th>Blood pressure (mm Hg)</th>
<th>Red blood cell number (10^6/\text{mm}^3)</th>
<th>Volume (fl)</th>
<th>Hematocrit (%)</th>
<th>Hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>35(14–65)</td>
<td>166(103)</td>
<td>4.92 ± 0.11</td>
<td>89.5 ± 0.9</td>
<td>44.1 ± 0.8</td>
<td>14.6 ± 0.2</td>
</tr>
</tbody>
</table>

\( p \) Value

NS <0.001 NS NS

Glomerulonephritis normotensive men

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yr)</th>
<th>Blood pressure (mm Hg)</th>
<th>Red blood cell number (10^6/\text{mm}^3)</th>
<th>Volume (fl)</th>
<th>Hematocrit (%)</th>
<th>Hemoglobin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>38(16–63)</td>
<td>123(76)</td>
<td>4.94 ± 0.06</td>
<td>85.8 ± 0.5</td>
<td>42.4 ± 0.6</td>
<td>14.3 ± 0.1</td>
</tr>
</tbody>
</table>

\( p \) Value

NS <0.001 NS NS

Values are means ± SEM, except for age and blood pressure, which are mean values. Numbers in parentheses indicate extreme values. NS = not significant.

Table 3. Platelet Indexes in Essential Hypertensive Patients and Normotensive Control Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Age (yr)</th>
<th>Blood pressure (mm Hg)</th>
<th>Platelet volume (fl)</th>
<th>Platelet count (10^9/\text{mm}^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential hypertensive men (Group 3)</td>
<td>47</td>
<td>45(32–61)</td>
<td>172(105)</td>
<td>9.39 ± 0.08*</td>
<td>286 ± 9</td>
</tr>
<tr>
<td>Normotensive men (Group 4)</td>
<td>42</td>
<td>41(25–59)</td>
<td>122(81)</td>
<td>9.04 ± 0.08</td>
<td>295 ± 8</td>
</tr>
<tr>
<td>Essential hypertensive women (Group 3)</td>
<td>36</td>
<td>46(27–58)</td>
<td>167(103)</td>
<td>9.48 ± 0.10f</td>
<td>306 ± 10</td>
</tr>
<tr>
<td>Normotensive women (Group 4)</td>
<td>23</td>
<td>39(23–56)</td>
<td>121(79)</td>
<td>9.15 ± 0.11</td>
<td>302 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SEM, except for age and blood pressure, which are mean values. Numbers in parentheses indicate extreme values.

\*p < 0.005, \( \dagger \)p < 0.05, compared with values in their respective control groups.

Filtration rate failed to exhibit the pattern of erythrocyte abnormalities observed in essential hypertension (see Table 2). In fact, the RBCV was higher in hypertensive GN patients than in normotensive GN patients matched for histological diagnosis, age, sex, and glomerular filtration rate. This difference is mainly due to a lower RBCV in normotensive GN patients than in normotensive healthy subjects.
The relationship of these blood cell defects to hypertension, as well as the relationship of these changes to each other, is not known. Clearly, these data point to some alteration of cell volume regulation, but they allow no speculation about its mechanisms. Alterations of cell volume were also found in vascular smooth muscle cells of SHR at different ages. Unfortunately, even basic knowledge about how cells regulate their volume is incomplete. It is interesting to note that two ionic systems involved in cell volume regulation, namely, a loop diuretic-sensitive Na⁺-K⁺ symport and intracellular calcium, have also been reported to be altered in hypertension. Increasing intracellular calcium with ionophores makes red blood cells shrink their volume (our unpublished observations, 1986).

One question arising is whether these hematological alterations somehow represent a consequence of high blood pressure. This possibility is suggested by the parallel, age-related course of hypertension and blood cell defects in SHR (see Figure 1). In contrast with this assumption are the observations that none of the three aforementioned hematological changes was observed in three different forms of secondary hypertension—namely, glomerulonephritis hypertension, DOCA-salt hypertension (see Tables 1 and 2), and renovascular hypertension—characterized by very high blood pressure and, presumably, by different mechanisms. On the other hand, a decrease in RBCV is present in another model of primary hypertension, the Milan hypertensive rat strain. Bianchi and colleagues showed that the RBCV abnormality is independent of the hypertensive environment, as the bone marrow of the Milan hypertensive rat strain still produces smaller erythrocytes even when transplanted into normotensive recipients.

The main purpose of the present study was to help identify elementary and quantitative parameters to compare spontaneous and essential hypertension and thus to face a part of the more general question of whether genetic hypertension is associated with the same biological background in different species. Setting this issue would implicitly indicate whether rats with spontaneous hypertension can be used as a copy of the human disease. The results of this and previous work suggested to us that this may be the case. The alteration of cytoplasmic calcium and of adrenergic receptors can be added to the three hematological indexes described in the present report, giving a complex of five parameters that are consensually changed in essential and spontaneous hypertension. The possibility that such consensuality is due to chance seems very limited, although the causal link between blood cell alterations and hypertension is unclear. Blood cell defects were found to be qualitatively similar in rat and humans, though quantitatively more prominent in the former. This greater prominence may result from a greater concentration of genetic factors in SHR because of continued inbreeding or to confounding environmental conditions that are easily controlled in captive animals. Alternatively, SHR might be repre-
sentative of only one subgroup of essential hypertensive patients. Their exaggerated patterns may prove useful if it could be demonstrated that they predict the changes occurring to a minor degree in (or in a minority of) essential hypertensive patients.

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Similarities of essential and spontaneous hypertension. Volume and number of blood cells.
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