Body fluid volumes in the spontaneously hypertensive rat

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Summary
1. Plasma volume, packed cell volume (PCV), blood volume, extracellular fluid volume (ECFV) and Evans blue disappearance rate were measured in conscious spontaneously hypertensive rats and in weight-matched Wistar normotensive rats.

2. Over the weight range studied (250–350 g), plasma and blood volumes were significantly lower in the spontaneously hypertensive rat. Extracellular fluid volumes were similar in the two groups. PCV and Evans blue disappearance rates were significantly higher in the spontaneously hypertensive rat.

3. Negative correlations were obtained between plasma volume and mean arterial pressure and between the plasma/interstitial fluid volume ratio and mean arterial pressure.

4. The normal extracellular fluid volume and the lack of correlation with mean arterial pressure excludes volume expansion as a pressor mechanism during the established phase of hypertension in the spontaneously hypertensive rat.

Key words: blood pressure, blood volume, extracellular volume, microvascular permeability, spontaneously hypertensive rat.

Abbreviations: ECFV, extracellular fluid volume; PCV, packed cell volume.

Introduction
Blood volume is an important determinant of cardiac output and blood pressure [1]. It is not surprising, therefore, that increased research interest has been directed towards the role of volume expansion in hypertension [2].

In hypertensive man, the status of the body fluid volumes is not clear [3]. Extracellular fluid volume (ECFV) has been reported to be increased [4, 5] or normal [6–8]. Plasma volume has been reported to be normal [4, 9–13] or increased [14]. The diversity in findings may be attributed to: differences in experimental technique, selection of subjects, lack of standardized procedure or choice of a reference frame [3].

The spontaneously hypertensive rat is generally accepted as an experimental model of essential hypertension. It was the purpose of this study to determine the body fluid volumes during the chronic phase of hypertension in this model.

Methods
Protocol
The choice of an appropriate normotensive control for the spontaneously hypertensive rat is difficult [15]. It is known that the Wistar–Kyoto rats are genetically different from the spontaneously hypertensive rat. In the selection of hypertensive and normotensive rats by selective inbreeding, it is probable that ‘normal’ characteristics would be lost in the abnormal strains, the spontaneously hypertensive rat and the Wistar–Kyoto normotensive rats. Therefore, any comparison with normality in studies on the spontaneously hypertensive rat would have to be made against the parent strain [15]. Our inbred
Wistar rats are bred in an identical manner to the spontaneously hypertensive rat colony. Seven month old spontaneously hypertensive rats and weight-matched normotensive rats were maintained on Purina chow and tap water. Carotid cannulation was performed 8 h before blood pressure and volume determinations were made. Arterial pressure was measured in the conscious rat through the carotid catheter [16].

Experiment 1

Volume determinations were made in the conscious rat by the dye-dilution method [17]. Plasma volume was measured by using Evans blue [18] and the thiocyanate space was used as an index of ECFV [19, 20]. Through the carotid catheter, 0.25 ml of a thiocyanate solution (0.81 mol/l; 25 mg) was injected. After 35 min, 0.20 ml of Evans blue solution (0.0156 mol/l; 3 mg) was injected. Each injection was followed by a 0.15 ml flush of NaCl solution (0.9%; 150 mmol/l) to clear the dye from the catheter and the blind segment of the common carotid artery. Five minutes after the injection of Evans blue, 1.0 ml of blood was withdrawn from the catheter, the packed cell volume (PCV) determined in duplicate and the blood sample centrifuged. Plasma (0.01 ml) was added to a cold solution of 0.80 ml of trichloroacetic acid (12.5%, w/v) and 1.0 ml of distilled water. The tubes were kept for 30 min and then centrifuged at 3500 rev/min for 30 min. To 0.80 ml of the supernatant, 0.80 ml of ferric nitrate solution (0.1238 mol/l; 40 mg) was added. The absorbance at 480 nm was determined. The thiocyanate space was calculated from: thiocyanate space (ml) = thiocyanate space - plasma volume.

Results

When compared with the American Wistar rat, for equivalent weight, plasma and blood volumes were decreased in the spontaneously hypertensive rat. In the spontaneously hypertensive rat the increase in PCV was associated with a reduced plasma volume. The ECFV was similar in the spontaneously hypertensive rat and the Wistar normotensive rat (Table 1). No correlation was observed between ECFV and the blood pressure (Fig. 1). A negative correlation was obtained between intravascular volume and blood pressure (Figs. 2, 3). The plasma/interstitial fluid volume ratio is an index of the partition of ECFV. There was a negative correlation between the plasma/interstitial fluid volume ratio and the blood pressure (Fig. 4). Evans blue disappearance rate was increased in the spontaneously hypertensive rats when compared with the Wistar normotensive rats (Table 2).

Discussion

Established hypertension in the spontaneously hypertensive rat is characterized by a normal ECFV and a lower intravascular volume than in weight-matched Wistar rats. A normal extra-
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**TABLE 1. Comparisons between the spontaneously hypertensive and normotensive control rats**

Values are means ± SD with numbers of rats shown in parentheses; N.S., not significant (P > 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Spontaneously hypertensive</th>
<th>Normotensive control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>166 ± 22 (22)</td>
<td>117 ± 13 (30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma volume (ml)</td>
<td>9.63 ± 1.3 (22)</td>
<td>11.3 ± 2.0 (27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(ml/100 g)</td>
<td>3.50 ± 0.26</td>
<td>4.03 ± 0.41</td>
<td></td>
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<tr>
<td>PCV (%)</td>
<td>48.5 ± 2.9 (22)</td>
<td>45.0 ± 1.9 (30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood volume (ml)</td>
<td>18.1 ± 2.0 (22)</td>
<td>19.8 ± 3.4 (27)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(ml/100 g)</td>
<td>6.46 ± 0.65</td>
<td>7.04 ± 0.68</td>
<td></td>
</tr>
<tr>
<td>ECFV (ml)</td>
<td>87.3 ± 18 (22)</td>
<td>81.0 ± 20 (29)</td>
<td>N.S.</td>
</tr>
<tr>
<td>(ml/100 g)</td>
<td>31.0 ± 4</td>
<td>28.8 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>Plasma/interstitial fluid volume ratio</td>
<td>0.139 ± 0.019 (22)</td>
<td>0.165 ± 0.038 (27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>279 ± 78 (22)</td>
<td>281 ± 31 (30)</td>
<td>N.S.</td>
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</tbody>
</table>

**TABLE 2. Evans blue disappearance rate in spontaneously hypertensive and normotensive control rats**

Values are means ± SD; numbers of rats are shown in parentheses; N.S., not significant (P > 0.05).

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>172 ± 28</td>
<td>115 ± 12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Evans blue disappearance rate (%/h)</td>
<td>31.3 ± 4.07</td>
<td>17.8 ± 4.80</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>296 ± 20</td>
<td>295 ± 17</td>
<td>N.S.</td>
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**FIG. 1.** Lack of relationship between ECFV and the blood pressure in normotensive (O) and spontaneously hypertensive (●) rats. n = 51; r = -0.18; not significant.
Fig. 2. Inverse relationship between the plasma volume and blood pressure in normotensive (○) and spontaneously hypertensive (●) rats. \( n = 49; r = -0.52; P < 0.01. \)

Fig. 3. Inverse relationship between the blood volume and blood pressure in normotensive (○) and spontaneously hypertensive (●) rats. \( n = 49; r = -0.41; P < 0.01. \)
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**FIG. 4.** Inverse relationship between the plasma/interstitial fluid volume ratio and the blood pressure in normotensive (○) and spontaneously hypertensive (●) rats. \( n = 49; r = -0.47; P < 0.01. \)

...cellular fluid volume has been previously described in this model [22]. In the New Zealand rat strain of genetic hypertension, a reduced extracellular fluid volume and exchangeable sodium has been reported [21]. Therefore, a reduced capacity of the kidneys to excrete salt and water in proper relation to intake and a consequent expansion of the ECF compartment [23] does not appear to be the mechanism of blood pressure elevation in rat models of genetic hypertension.

Plasma volume has been reported as normal in the spontaneously hypertensive rat [22] and reduced in the New Zealand rat strain of genetic hypertension [21]. In the present study, in addition to a reduced plasma volume, we noted a negative relationship between the plasma volume and the blood pressure. A reduced plasma volume and a negative relationship between blood pressure and plasma volume has also been described in two-kidney renal hypertension in the rat [24]. In the presence of an intact kidney that is exposed to the systemic blood pressure, the reduction of intravascular volume may be considered a pressure-related phenomenon. In essential hypertension a negative linear [25] and an inverse hyperbolic relationship [26] between intravascular volume and blood pressure has been reported. The negative linear relationship has been attributed to an increased transcapillary escape rate of albumin [25, 27]. The inverse hyperbolic pattern has been suggested to result from loss of the pressure-body fluid control mechanism and consequently to a renal defect in hypertension [1, 26]. Although the kidney can respond to changes in the body fluid volumes, we consider the renal defect in hypertension, if any, to be more closely related to the intravascular volume, since the extracellular compartment remains unchanged. During the initial phase of hypertension, stroke-prone spontaneously hypertensive rats retain more sodium than normotensive Wistar-Kyoto rats of the same age [28]. Fractional sodium excretion and blood pressure rise with age and, whereas sodium balance is in equilibrium at 4 months, it becomes negative in the established phase of hypertension. During the development of hypertension intravascular volume of young stroke-prone spontaneously hypertensive rats is diminished despite sodium retention, indicating fluid depletion. The different renal handling of sodium and water suggests that the stroke-prone rat can excrete water but can eliminate sodium only if the blood pressure is sufficiently elevated. We agree with their findings that an expansion of...
the intravascular volume is not necessarily the first step in the mechanism of chronic blood pressure elevation [28].

However, an alternative explanation is possible. In the presence of hypertension, a reduced intravascular capacity and a pressure-related decrease in intravascular volume may represent independent variables determining the volume-capacity relationship within the cardiovascular system. The spontaneously hypertensive rat exhibits rarification of blood vessels in various circulatory beds, even in the prehypertensive state, including the cremasteric muscle [29] and the mesentery [30], and this may contribute to the decrease in vascular capacity and volume. It may therefore be argued that, despite a reduced intravascular volume, a greater decrease in capacity (in relation to volume) would represent 'inappropriate' hypervolaemia. We would, however, prefer to agree with Swales [31] and state that the best measurement of capacity is the determination of volume in absolute units. Without a change in capacity, a structurally reduced compliance of the capillary vessels alone [32] cannot influence intravascular volume.

There is an increased PCV in the spontaneously hypertensive rat [31] and in the New Zealand rat strain of genetic hypertension [21]. In the spontaneously hypertensive rat, Sen et al. [33] provided evidence that the higher PCV resulted from erythrocytosis. In their study, plasma volumes determined at 20 min after the injection of radio-iodinated albumin were found to be identical with those of normotensive rats. In our study, plasma volumes determined at 5 min were lower in the spontaneously hypertensive rat and we agree with Gresson et al. [21] in that the higher PCV in rat models of genetic hypertension is related to a lower plasma volume.

An increased disappearance rate of Evans blue occurs in hypertensive man and has been attributed to an abnormality in mixing of the labeling, a capillary defect or an increased rate of protein exchange between the intravascular and interstitial compartments [27]. There is an increased disappearance rate of Evans blue in the spontaneously hypertensive rat and we agree with Rippé et al. [34] that this probably reflects an increased capillary hydrostatic pressure. In the New Zealand rat strain of genetic hypertension, however, an increased disappearance rate of the labelled protein was not observed [21]. This may represent a difference between the two hypertensive rat strains.

A reduced plasma/interstitial fluid volume ratio represents an abnormality of partition of ECFV. Although we noted a reduced ratio in the spontaneously hypertensive rat, Trippodo et al. [22] did not detect a difference in the same strain when studied at 18–43 days. In the present study, adult rats with severe and long standing hypertension were examined. A reduction in this ratio may therefore be considered a function of the severity and/or duration of blood pressure elevation. Tarazi et al. [7] and Ibsen & Leth [12] found a reduction of the plasma/interstitial fluid volume ratio in men with essential hypertension. This defect in the partition of the ECFV may be related to either an increased transcapillary escape rate [25, 27] or an increased venous resistance [32, 35]. It is interesting to note, however, that an increased plasma/ECFV ratio was described by Lucas & Floyer in renal-prival and one-kidney renal hypertension in the rat [17, 36]. In these situations, a decreased secretion of a renal hormone, capable of altering interstitial tissue compliance, was proposed to explain the altered body fluid distribution.

In the spontaneously hypertensive rat, elevation of the blood pressure in the presence of an unchanged ECFV, a reduced blood volume and an increased disappearance rate of albumin from the intravascular compartment indicate that factors determining intravascular volume, capillary hydrostatic pressure and permeability are important in the pathogenesis of hypertension.

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