PLASMA VOLUME, EXTRACELLULAR FLUID VOLUME AND EXCHANGEABLE SODIUM CONCENTRATIONS IN THE NEW ZEALAND STRAIN OF GENETICALLY HYPERTENSIVE RAT

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SUMMARY

1. Rats of the New Zealand strain with genetic hypertension (GH rats) were found to have lower plasma volume in relation to body weight than rats of the normotensive parent strain (N rats). GH rats had higher venous packed-cell volume percentage than N rats, but total erythrocyte volume was similar in the two strains of rats. Extracellular fluid volume and exchangeable sodium concentrations were lower in the GH rats. Plasma sodium concentration was not altered; plasma potassium concentration was slightly higher in the GH rats.

2. These results indicate that hypersecretion of a sodium-retaining hormone is unlikely to be a primary factor causing hypertension in the GH rats. Cardiac output has not been measured: if an increased cardiac output is a factor in the maintenance of the hypertension, it is evidently not secondary to an increased blood volume.

3. The lower exchangeable sodium concentrations and body fluid volumes found in the GH rats may be due to lower aldosterone activity secondary to a decrease in the activity of the renin–angiotensin system, or to the effects of the elevated blood pressure on the renal handling of sodium, or to both factors.

Key words: genetically hypertensive rats, plasma volume, venous packed-cell volume, extracellular fluid volume, exchangeable sodium.

The development of a strain of albino rats with genetic hypertension (GH rats) has been described by Smirk & Hall (1958) and more recently by Phelan (1968). The cause of the hypertension of the GH rats is largely unknown. However, McKenzie & Phelan (1969) found lower plasma and renal renin concentration in the GH rats than in a random-bred strain of normotensive control (N) rats, and further studies (Gresson, 1972a) on renal renin concentration in the two strains have confirmed the lower concentrations in the GH rats. It would seem that overactivity of the renin–angiotensin system does not play a primary role in the maintenance of the hypertension of the GH rats.

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The present studies were designed to investigate factors relevant to the control of blood pressure and thus to build up a picture of the balance of factors maintaining the higher blood pressure in the GH rats.

**METHODS**

Male, albino rats were used throughout this study: the New Zealand inbred strain of genetically hypertensive (GH) rat, originally of Wistar stock, and for control purposes the random-bred parent strain of normotensive (N) rats from which the breeders, ancestral to the GH strain, were obtained in 1955 (Phelan, 1968). All rats were fed a pelleted diet containing approximately 0.5% (w/w) NaCl, and tap water *ad libitum*. The rats were allowed free access to food and water up to the time of measurement of plasma volume, extracellular fluid volume and exchangeable sodium concentrations. All values given, where applicable, are the means ±1 standard deviation (SD).

**Plasma volume, venous packed-cell volume percentage (PCV) and blood pressure (BP)**

Plasma volume and venous PCV were measured in a total of sixty GH rats and sixty N rats over an extensive range of age and weight. In addition, in some instances plasma volume was measured in the same rats at different ages: the minimum time between successive measurements was 7 weeks. Thus the total number of plasma volume measurements was about eighty in both groups of rats.

As an additional control group the venous PCV was measured in ten black rats of an unrelated normotensive strain, aged 13 weeks (BP 105 ± 8 mmHg). The venous PCV of five 3-week-old N rats was also measured.

Plasma volume was measured as described by Belcher & Harriss (1957) except that jugular veins were used for injection and sampling. Rats were anaesthetized with ether [Wang (1959) found that ether anaesthesia had little, if any, effect on plasma volume in the rat] and both jugular veins exposed; 0.2 ml of a 0.5% (w/v) Evans Blue solution was injected slowly into the left jugular vein (Renaud, 1969) and after 5 min a 0.6 ml blood sample was taken from the right jugular vein. The extinction of the plasma sample was measured at a wavelength of 605 nm in a spectrophotometer, and the concentration of Evans Blue estimated by reference to a calibration curve. Plasma volume was estimated by standard dilution theory, equilibrium in the distribution of the tracer being assumed:

\[
\text{Volume of distribution of tracer} = \frac{\text{Amount of tracer injected}}{\text{Concentration of tracer in sample}}
\]

To minimize the effects of diurnal variation all plasma volumes were measured between 09.00 and 13.00 hours.

The PCV of venous blood was estimated after centrifugation at 1000 g for 60 min of a blood sample taken in a capillary tube; all PCV values were corrected by a factor of 0.96 to allow for ‘trapped plasma’ (Leeson & Reeve, 1951).

Systolic blood pressure was measured under light ether anaesthesia at least 1 day before the plasma volume was measured, by means of a modification of the tail-cuff method described by Dowd & Jones (1968).

**Rate of loss of Evans Blue from the circulation**

In the measurement of plasma volume a standard 5 min equilibration time was used, rather
Genetically hypertensive rats

than serial blood sampling with extrapolation of Evans Blue concentration to zero time. The use of a single blood sample for measurement of plasma volume in the rat has been justified by Belcher & Harriss (1957). To determine whether the rate of loss from the circulation differed in the GH and N rats Evans Blue was injected, as described above, into eight GH rats (264±6 g; BP 159±9 mmHg) and eight N rats (265±13 g; BP 112±4 mmHg), and its concentration measured in plasma samples after 5 min, 3 h and 6 h. The concentration of Evans Blue after a circulation time of 5 min was taken as 100% and the percentage decrease after circulation times of 3 h and 6 h was calculated. Measured concentrations were corrected for the total amount of Evans Blue removed in previous samples (Belcher & Harriss, 1957). The rate of loss of Evans Blue from the circulation was similar in the two strains of rat. After 3 h the decrease in Evans Blue concentration in the plasma of the GH rats was 36·1±3·2% and in the N rats 35·8±1·5%. After 6 h the decrease was 51·4±2·0% and 51·8±2·0% for the GH and N rats respectively.

**Total erythrocyte volume**

Total erythrocyte volume was measured in twelve GH rats (aged 4–5 months) and in twelve weight-matched N rats (aged about 3 months) by means of 51Cr-labelled erythrocytes. Blood pressure had been measured in the rats at least 1 day before measurement of total erythrocyte volume.

Erythrocytes were labelled with 51Cr by incubating whole blood in a citrate–dextrose solution (Ebaugh, Emerson & Ross, 1953) containing approx. 50 μCi of 51Cr (as sodium chromate: The Radiochemical Centre, Amersham). After incubation the plasma was separated and discarded, and the cells were washed twice in Ringer's solution (NaCl 147 mM, KCl 4·2 mM, CaCl2,2H2O 2·8 mM) to remove unbound 51Cr. The cells were resuspended in Ringer's solution and this suspension was used for injection into the rats. Erythrocytes were labelled with 51Cr immediately before each experiment. Blood from a GH rat was used for labelling and injection into the GH rats; similarly, blood from an N rat was used for injection into the N rats.

Rats were anaesthetized with ether and 0·2 ml of labelled 'blood' (approx. 0·8–1 μCi of 51Cr) was injected into the right jugular vein; 5 min later 0·2 ml Evans Blue (1 mg) was injected into the left jugular vein and 5 min after this a blood sample was withdrawn from the right jugular vein. Complete equilibration of 51Cr-labelled cells in the circulation of the rat occurs by 10 min (Belcher & Harriss, 1957; Rettori, Mejia & Fernandez, 1964). Whole blood samples were counted in a Packard Auto-Gamma spectrophotometer for 51Cr activity, and Evans-Blue plasma concentration was measured as described earlier. Total erythrocyte volume and plasma volume were calculated by standard dilution theory, and were summed to give total blood volume.

**Extracellular fluid volume**

Extracellular fluid volume (ECFV) was measured in twenty GH rats (aged 4–7 months) and in twenty weight-matched N rats (aged 3–6 months) by means of inulin. The blood pressure of the rats had been measured at least 1 day before ECFV was measured.

For measurement of ECFV rats were anaesthetized with ether and both kidneys exposed by a dorsal incision. The renal pedicles were ligated to prevent renal excretion of inulin. The left jugular vein was then exposed and 0·15 ml of a sterile inulin solution (3 mg of inulin) was injected. After 1 h the rats were again anaesthetized, the abdominal aorta was exposed and a
6 ml blood sample taken. Trichloroacetic acid (20% solution, w/v) was added to the plasma (1:1, v/v) to precipitate proteins and the concentration of inulin in the supernatant was measured as described by Heyrovsky (1956) with slight modification. After hydrolysis the extinction of the resulting fructose–indolylacetic acid complex was measured at a wavelength of 515 nm and inulin concentration estimated by reference to a calibration curve. ECFV was estimated by standard dilution theory.

In N rats we found that the volume of distribution of inulin was no greater after 120 min than after 60 min.

Exchangeable sodium concentrations

Exchangeable sodium (Naₐ) concentrations were measured in fifteen GH rats (aged 4–6 months) and in fifteen weight-matched N rats (aged 3–6 months) by means of an isotope-dilution technique. The blood pressure of the rats had been measured at least 1 day before Naₐ was measured.

For measurement of Naₐ the rats were anaesthetized with ether and 0.25 ml of ²²NaCl solution (The Radiochemical Centre, Amersham), containing approximately 2 μCi of ²²Na was injected into the left jugular vein. After 24 h the rats were placed in a large-volume gamma counter and total-body radioactivity was measured. Immediately afterwards a 1 ml blood sample was taken from the right jugular vein and the specific radioactivity of the plasma was measured. Counts of total body radioactivity and of specific radioactivity of the plasma were corrected to the same efficiency by reference to an external standard. Naₐ was then estimated by dividing total-body radioactivity by the specific radioactivity of sodium in the plasma.

Plasma electrolytes

In a separate series of investigations plasma sodium and potassium concentrations were measured by flame photometry in thirty-six adult GH rats (aged 3–6 months) and in thirty-six adult N rats (aged 3–6 months): a lithium-based heparin (Sigma) was used.

RESULTS

Plasma volume and venous PCV

The GH rats had a lower plasma volume over the range of body weight from about 150–300 g (Fig. 1). They had a higher venous PCV than the N rats at all ages (Fig. 2). Polynomials of 'best-fit' in Figs. 1–3 were calculated by computer analysis. An analysis of co-variance showed that the difference in plasma volume between the two strains of rats was highly significant \( F = 28.76, P < 0.001 \), as was the difference in PCV \( F = 55.29, P < 0.001 \). The PCV values of ten normotensive black rats were very similar to the PCV of the normotensive albino (N) rats (Fig. 2).

Throughout the age-range studied, blood pressure was higher in the GH rats than in the N rats (Fig. 3).

To elucidate possible relationships between blood pressure, plasma volume, and venous PCV a multiple correlation analysis was carried out. In neither group of rats was a significant relationship between blood pressure and plasma volume found. There was also no significant relationship between blood pressure and venous PCV. However, in both groups of rats plasma volume and venous PCV were negatively related: partial correlation coefficients of \(-0.396 (P < 0.001)\) and \(-0.276 (P < 0.05)\) were obtained for the GH and N rats respectively.
Genetically hypertensive rats

Fig. 1. Plasma volume related to total body weight in genetically hypertensive rats (▲) and normotensive rats (○). Polynomials of 'best-fit' are shown.

Fig. 2. Venous packed-cell volume percentage in genetically hypertensive rats (▲), white normotensive rats (○), and black normotensive (■) rats. Polynomials of 'best-fit' are shown; the values for the black normotensive rats were not included in the regression analyses.
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FIG. 3. Mean systolic blood pressure in genetically hypertensive rats (▲) and normotensive rats (○). Polynomials of 'best-fit' are shown.

**Total erythrocyte volume**

The GH rats had a mean total erythrocyte volume very similar to the value found in the N rats (Table 1). Plasma volume was significantly lower in the GH rats and consequently total blood volume was also lower (Table 1). Reciprocally, the venous PCV was significantly higher in the GH rats.

**TABLE 1. Total erythrocyte volume, plasma volume (PV) and blood volume (BV) in genetically hypertensive (GH) and normotensive control (N) rats**

<table>
<thead>
<tr>
<th></th>
<th>GH (n = 12)</th>
<th>N (n = 12)</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte volume (ml/100 g)</td>
<td>2.00 ± 0.12</td>
<td>2.08 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>PV (ml/100 g)</td>
<td>3.85 ± 0.16</td>
<td>4.20 ± 0.17</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>BV (ml/100 g)</td>
<td>5.86 ± 0.16</td>
<td>6.28 ± 0.22</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>Venous PCV (%)</td>
<td>49.0 ± 2.6</td>
<td>47.0 ± 1.7</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>269 ± 11</td>
<td>275 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>174 ± 5</td>
<td>107 ± 11</td>
<td>(P &lt; 0.001)</td>
</tr>
</tbody>
</table>

The results are means ±1 SD. \(P\) values were derived from Student's t-test. This was a separate group of animals, and the results are not incorporated in Figs. 1-3. NS = not significant \((P > 0.05)\).
Extracellular water volume (ECFV)

The GH rats had a significantly lower ECFV than N rats, a mean difference of 2.0 ml/100 g body weight being found (Table 2). In neither strain of rats was a significant correlation between blood pressure and ECFV (expressed as ml/100 g body weight) found.

### Table 2. Extracellular fluid volume (ECFV) in genetically hypertensive (GH) and normotensive control (N) rats

<table>
<thead>
<tr>
<th></th>
<th>GH (n = 20)</th>
<th>N (n = 20)</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECFV (ml/100 g)</td>
<td>14.3 ± 1.0</td>
<td>16.3 ± 1.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>292 ± 29</td>
<td>290 ± 32</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>175 ± 9</td>
<td>110 ± 10</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

The results are means ± 1 SD. P values were derived from Student's t-test. NS = not significant (P > 0.05).

Exchangeable sodium (Na\textsubscript{e}) and plasma electrolyte

The GH rats had significantly lower Na\textsubscript{e} concentrations than N rats, the mean difference being 4.0 mmol/kg (Table 3). We were not able to find a significant correlation between blood pressure and Na\textsubscript{e} levels (expressed as mmol/kg body weight) in either group of rats.

### Table 3. Exchangeable sodium (Na\textsubscript{e}) values in genetically hypertensive (GH) and normotensive control (N) rats

<table>
<thead>
<tr>
<th></th>
<th>GH (n = 15)</th>
<th>N (n = 15)</th>
<th>Significance of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na\textsubscript{e} (mmol/kg)</td>
<td>40.5 ± 1.8</td>
<td>44.5 ± 1.9</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>279 ± 17</td>
<td>44.5 ± 1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>193 ± 17</td>
<td>122 ± 6</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

The results are means ± 1 SD. P values were derived from Student's t-test. NS = not significant (P > 0.05).

No difference was found in plasma sodium concentration: mean values of 141.5 ± 6.4 mmol/l and 140.3 ± 7.3 mmol/l were found in the GH and N rats respectively. Plasma potassium concentration was significantly higher by a mean of 0.4 mmol/l in the GH rats; mean values of 4.2 ± 0.5 mmol/l and 3.8 ± 0.4 mmol/l (P < 0.001) were found in the GH and N rats respectively. When the total amount of extracellular potassium (in solution) was estimated by reference to the ECFV, mean values of 0.60 mmol/kg body weight and 0.62 mmol/kg were found in the GH and N rats respectively. It seems probable that despite the higher plasma potassium concentration there is no increase in total extracellular potassium in the GH rats.
DISCUSSION

The results show that the GH rats have lower body fluid volumes and lower Na⁺ concentrations than do rats of the normotensive parent strain; the GH rats also have lower total carcass sodium content (Gresson, 1972a). The findings argue against involvement of aldosterone, or other sodium-retaining hormones, as a factor in the hypertension of the GH rats. The data are compatible with a lower level of aldosterone activity in the GH rats, perhaps secondary to the lower activity of the renin–angiotensin system (McKenzie & Phelan, 1969; Gresson, 1972a). The high arterial blood pressure may be the cause of the suppression of activity of the renin–angiotensin system in the GH rats. It would seem that as a consequence of the hypertension the activity of the renin–angiotensin–aldosterone system is decreased in the GH rats and that this, along with possible effects of the hypertension on the renal handling of sodium, leads to lower Na⁺ concentrations and consequently to lower body fluid volumes. It appears, in fact, that, if these differences between the GH and N rats are related to the hypertension, then they are secondary to it.

Our suggestion that plasma volume, ECFV and Na⁺ are lower in the GH rats as a consequence of the hypertension would have been strengthened by finding an inverse relationship between blood pressure and these parameters within the various groups of GH rats investigated. Failure to find such a relationship may be due, in part, to the lability of blood pressure in the GH rats. With regard to plasma volume, it is apparent that the difference between the two strains of rats increased progressively from a mean estimated difference of 0.31 ml at body weight 100 g to 0.70 ml at body weight 250 g (Fig. 1). In older rats the difference in plasma volume lessens (Fig. 1). Recently (C. R. Gresson, unpublished work) we have found that, in many instances, old GH rats have greater plasma volume than N rats; this seems to coincide with the development of vascular damage in the GH rats.

The higher venous PCV found in the GH rats (Fig. 2) cannot readily be explained on the basis of a primary polycythaemia: total erythrocyte volume was similar in adult rats of the GH and N strains (Table 2). The difference in PCV could be due to a difference in the strain of rat, unrelated to the hypertension; however, the venous PCV of black normotensive rats was very similar to values found in the albino normotensive (N) strain (Fig. 2). The significant negative correlation between plasma volume and PCV found in both strains of rats supports the suggestion that the higher PCV found in the GH rats is due, at least in part, to lower plasma volume. Arterial PCV is also higher in the GH rats (Gresson, 1972b) although the difference between the two strains is less than is the venous PCV difference. The higher venous PCV found in the 3-week-old GH rats (Fig. 2) may indicate a lower plasma volume even at this age, but this requires further investigation. Thus in GH rats, at least before secondary vascular damage has occurred, plasma volume and probably also total blood volume are not increased above values found in N rats of similar body weight. It is unlikely, therefore, that the hypertension of the GH rats is, at this stage, maintained by increased cardiac output secondary to increased blood volume.

GH rats tend to be lighter in weight than N rats of similar age. Weight-matching is essential before making valid comparisons of parameters such as plasma volume which, when expressed as ml/100 g body weight, shows a strong negative regression on body weight; other groups of workers have also found this negative regression in the rat (Belcher & Harriss, 1957; Constable, 1963; Rettori et al., 1964). We have found that ECFV (expressed as ml/100 g body weight)
Genetically hypertensive rats and Na\(_a\) (expressed as mmol/kg body weight) also tend to correlate inversely with body weight. Weight-matching between GH and N rats has meant that the GH rats used in the experiments were older than the N rats, but this was largely unavoidable. In the case of the venous PCV the difference between the two strains of rats was evident over an extensive age range, and it would seem unlikely that the lower body fluid volumes and Na\(_a\) values found in the GH rats in comparison to N rats are due to the age discrepancy.

In a separate series of investigations we found the fat content of GH rats to be less than that of N rats; expression of plasma volume, ECFV and Na\(_a\) in terms of lean rather than total body weight increases the difference found between the two strains.

Plasma protein concentration is higher in the GH rats (Gresson, 1972b); this and the higher venous PCV found in the GH rats (and also higher arterial PCV: Gresson, 1972b) indicate a higher blood viscosity (Begg & Hearns, 1966; Schrier, McDonald, Wells & Lauler, 1970; Zingg, Sulev & Morgan, 1970) which may have a slight effect on blood pressure.

It is of interest that in the Japanese strain of spontaneously hypertensive (SH) rats (Oka-moto & Aoki, 1963), plasma volume is similar to values found in a strain of normotensive control rats (Sen, Hoffman, Stowe, Smeby & Bumpus, 1972) and plasma potassium concentration is lower (Nagaoka, Kikuchi & Aramaki, 1970; Baer, Knowlton & Laragh, 1972). The latter finding might be a consequence of hypersecretion of aldosterone, as found by Rapp & Dahl (1971). Thus the aetiology and/or pathogenesis of the hypertension in the SH and GH rats may well differ. In the SH rats it seems possible that mineralocorticoids are playing a larger role in the hypertension than they do in the GH rats.

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