Plasma and interstitial volumes in essential hypertension: relationship to blood pressure

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Summary

1. Plasma and interstitial fluid volumes have been measured simultaneously in men with uncomplicated and untreated essential hypertension.
2. Plasma volume (Evans blue) was reduced in essential hypertension and correlated inversely with blood pressure.
3. Interstitial fluid volume, derived from bromide space and plasma volume, was also reduced in essential hypertension and correlated inversely with blood pressure. The mean reduction in both plasma and interstitial fluid volumes was 6–7%.
4. There is no evidence for volume expansion in essential hypertension and the relationship between plasma and interstitial fluid volumes is preserved. The contraction of plasma and interstitial fluid volumes is most likely to reflect a natriuresis and diuresis secondary to the raised renal perfusion pressure, and sodium retention is unlikely to be a primary event in essential hypertension.

Key words: essential hypertension, interstitial fluid volume, plasma volume.

Introduction

In certain situations, such as advanced renal failure, hypertension is caused by sodium retention [1–4]. In other situations, such as mineral-corticoid excess, hypertension is associated with extracellular fluid volume expansion yet probably not caused by it [5–7]. In essential hypertension and renovascular hypertension exchangeable sodium has been reported as normal [6, 8–11] or increased [12–14]. This is surprising as increased perfusion pressure causes a natriuresis [15], which might be expected to result in sodium balance being negative. Conversely, it has been proposed on the basis of both experimental and clinical studies that the relationship between perfusion pressure and sodium excretion is altered in essential hypertension and therefore the expected sodium loss does not occur [16–18].

Despite the reported normality of exchangeable sodium, plasma volume has repeatedly been found to be reduced in essential hypertension [19–22] and to be inversely related to blood pressure [20, 22]. This would favour a normal pressure–natriuresis relationship. However, vascular permeability may be increased in hypertension [23], and the reduced plasma volume could theoretically reflect a redistribution of extracellular fluid.

We have accordingly measured oth plasma and extracellular fluid volumes in hypertensive and normotensive men and related these measurements to blood pressure.

Methods

Subjects

Sixty-eight male Caucasian subjects aged 20–65 years were studied, to avoid sex and racial differences. The hypertensive patients (41) were referred to the Hypertension Clinic for investigation and treatment; all had at least two casual outpatient blood pressure recordings...
greater than 140/90 mmHg and had received no antihypertensive medication. Full clinical assessment and investigation including urinalysis, plasma electrolytes, blood urea (<6.5 mmol/l), serum creatinine (<120 μmol/l), endogenous creatinine clearance (>80 ml/min), 24 h urinary vanillylmandelic acid and/or free noradrenaline and adrenaline excretion, chest X-ray, electrocardiogram and, in most instances, intravenous pyelography failed to identify any cause or complication of hypertension.

The normotensive control subjects (27, blood pressure <140/90 mmHg) consisted of ambulant patients admitted for minor orthopaedic procedures and medical staff. All were studied in an identical manner to the hypertensive patients. All hypertensive subjects were inpatients, and diet and physical activity were unrestricted except where stated. Informed consent was obtained before investigation and the following protocol was approved by the local medical ethical and MRC isotope advisory committees: day 1, admission; day 3, between 09.00 and 10.00 hours after overnight fasting and at least 1 h recumbent, measurement of plasma volume by using Evans blue [24]; day 4, measurement of extracellular fluid by using radioactive bromine (82Br) [25].

Blood pressures used in the subsequent analysis were the means of three recordings made with a standard sphygmomanometer (phases I and V) immediately before the determination of plasma volume. All investigations and measurements were carried out by the same individual.

**Plasma volume**

A weighed amount of a 5% (w/v) solution of Evans blue (Harvey Labs. Inc., Philadelphia, U.S.A.) was injected into a right antecubital vein. Blood samples were taken before and on four timed occasions, 5–8 min apart, after the injection of dye from an indwelling i.v. cannula in the left forearm. The blood samples were anticoagulated with lithium heparin, and after centrifugation plasma was stored at −20°C until assayed. The Evans blue concentration in the samples was determined spectrophotometrically by the method of Constable [26] using a Pye-Unicam SP 500 series 2 spectrophotometer. The initial plasma, before injection of dye, was used in each subject for blank and standards. Absorbance was read at 620 μm and a semilogarithmic plot was constructed of absorbance against time. The absorbance at zero time was calculated by extrapolation of the regression line and used to calculate plasma volume. Evans blue clearance was calculated from the half-life in plasma and volume of distribution.

**Erythrocyte volume**

This was calculated from plasma volume and whole-body packed cell volume. Peripheral venous packed cell volume was measured on the initial blood sample by using a Gelman–Hawksley microhaematocrit centrifuge. Corrections for trapped plasma were made according to the manufacturers’ instructions and for whole-body/peripheral venous packed cell volume ratio by a factor of 0.91 [27, 28].

**Extracellular fluid and interstitial fluid volumes**

These were calculated from the radioactive bromine space and plasma volume. 82Br (20–30μCi; The Radiochemical Centre, Amersham, Bucks., U.K.) was injected intravenously and a urine collection started. Six hours later a blood sample was taken from another vein and the urine collection completed. The radioactivity in the plasma, urine and a standard prepared from the solution injected was measured with an EKCO thorium-activated sodium iodide well scintillation-counter and Nuclear Enterprise scaler-ratemeter. The bromine space was calculated by the dilution principle after correction for urinary loss and bromine in erythrocytes (5% of radioactivity remaining in the body at 6 h [29]).

In 18 subjects bromine space was measured at both 3 and 6 h, to confirm that 6 h was an adequate equilibration period. The amount of bromine in erythrocytes was checked by measuring the difference in radioactivity between whole blood and plasma and by using packed cell volume and erythrocyte volume to calculate percentage of radioactivity in erythrocytes at 6 h. Extracellular fluid (ECF) and interstitial fluid (IF) were then derived from the following formulae [30] (1–1.1, Donnan effect).

\[
IF = \frac{\text{bromine space} - \text{plasma volume}}{1.11}
\]

\[
ECF = IF + (0.92 \times \text{plasma volume})
\]

The MRC isotope advisory committee restricted the number of normotensive subjects receiving 82Br to five and suggested that published data for predicting extracellular fluid should be used for comparison [31].

Statistical analysis was by Student’s paired and unpaired t-tests and correlation coefficients were calculated by the method of least squares.

**Results**

**Plasma volume**

The clinical data for the normotensive and hypertensive subjects studied are shown in Table.
TABLE 1. Clinical data
Results are means ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th>Essential hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 27)</td>
<td>(n = 41)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127 ± 2-3</td>
<td>161 ± 3-5</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>78 ± 1-0</td>
<td>102 ± 2-2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.1 ± 1-5</td>
<td>77.0 ± 1-1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175 ± 1-3</td>
<td>177 ± 4-3</td>
</tr>
<tr>
<td>Surface area (m²)</td>
<td>1.90 ± 0-02</td>
<td>1.91 ± 0-02</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.2 ± 2-9</td>
<td>46.7 ± 1-4*</td>
</tr>
<tr>
<td>Urinary Na excretion (mmol/day)</td>
<td>145 ± 14</td>
<td>138 ± 10</td>
</tr>
</tbody>
</table>

* P < 0.01.
† Reference [32].

TABLE 2. Correlation of plasma volume (L) with other parameters in normal subjects (n = 27)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area (m²)</td>
<td>0.919*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.860*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.701*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.224*</td>
</tr>
</tbody>
</table>

* P < 0.01; N.S., not significant.

1. The groups were well matched except for age. Urinary sodium excretion was also similar: normals 145 ± 14 mmol/day and hypertensive subjects 138 ± 10 mmol/day. In normal subjects plasma volume correlated with body surface area [32], body weight and height, but not with age (Table 2). However, plasma volume when expressed as a function of body surface area was still significantly correlated with body surface area (L/m² vs m², r = 0.69, P < 0.01), suggesting that not all variability had been eliminated. Similar findings with body weight [20] emphasize the importance of using regression analysis in comparing groups of subjects with different body builds. Therefore in the following analyses plasma volume is expressed as a percentage of that predicted by body surface area from the regression analysis of data obtained in normal subjects. Plasma volume in normal subjects was unrelated to age even when corrected for body build (% predicted, r = -0.26, P > 0.1).

Plasma volume in hypertensive patients was lower than in normal subjects with any of the above parameters, and packed cell volume was higher in hypertension and the calculated erythrocyte volume was the same in the two groups of subjects (Table 3).

The combined data for normal subjects and hypertensive patients showed a significant inverse relationship between plasma volume (% predicted by body surface area) and blood pressure: diastolic r = -0.36 (Fig. 1), systolic r = -0.34 (P < 0.01 for both).

Evans blue clearance was higher in hypertension, but not significantly so (2.8 ± 0.3 vs 2.3 ± 0.3 ml/min; P = 0.20).

Extracellular fluid and interstitial fluid volumes

These were measured in 30 individuals (five normal subjects). In 18 subjects bromine spaces were calculated at 3 and 6 h after the injection of 82Br. There was no significant difference in
that predicted 18.76 litres, and the observed value compared with a predicted volume of 18.76 ± 0.53 litres; P < 0.1, >0.05). In hypertensive patients extracellular fluid was significantly reduced (Table 4). This might simply reflect the lower plasma volume in hypertension. However, observed interstitial fluid volume (extracellular fluid – plasma volume) was also reduced in these subjects (Table 4, P < 0.02). In contrast, interstitial fluid in the five normal individuals was greater than predicted (observed 16.25 ± 0.38 litres, predicted 15.81 ± 0.45 litres; P < 0.025).

Interstitial fluid was inversely correlated with blood pressure: diastolic r = −0.53, systolic r = −0.48 (P < 0.01 for both). The relationships of plasma volume and interstitial fluid to diastolic blood pressure, including all 30 subjects, are shown in Fig. 2.

Discussion

Increased renal perfusion pressure results in a natriuresis and diuresis in the presence of normal renal function [15]. Therefore to maintain sodium balance in hypertension the relationship between sodium excretion and perfusion pressure must be shifted to the right [16, 17]. The question of whether this shift is the primary event in the pathogenesis of essential hypertension or occurs as a consequence of the hypertension remains unresolved. If the former were the case then the early phase would be associated with sodium retention and volume expansion, these abnor-

TABLE 4. Plasma volume, extracellular fluid and interstitial fluid in essential hypertension (n = 25)

<table>
<thead>
<tr>
<th></th>
<th>Observed (litres)</th>
<th>Predicted† (litres)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma volume</td>
<td>2.86 ± 0.05</td>
<td>3.09 ± 0.05**</td>
</tr>
<tr>
<td>Extracellular fluid</td>
<td>17.50 ± 0.34</td>
<td>18.19 ± 0.27**</td>
</tr>
<tr>
<td>Interstitial fluid</td>
<td>14.83 ± 0.31</td>
<td>15.34 ± 0.22*</td>
</tr>
</tbody>
</table>

† Reference [31].
* P < 0.02.
** P < 0.01.

The 82Br content of erythrocytes at 6 h was measured in 17 subjects and found to be 5.03 ± 0.33% of the counts remaining in the body. All subsequent calculations for extracellular fluid and interstitial fluid were therefore made after a 6 h equilibration period and allowing 5% for the 82Br in erythrocytes.

In normal subjects the mean value for extracellular fluid was not significantly different from that predicted [31] (19.26 ± 0.41 litres for the observed value compared with a predicted volume of 18.76 ± 0.53 litres; P < 0.1, >0.05). In hypertensive patients extracellular fluid was significantly reduced (Table 4). This might simply reflect the lower plasma volume in hypertension. However, observed interstitial fluid volume (extracellular fluid – plasma volume) was also reduced in these subjects (Table 4, P < 0.02). In contrast, interstitial fluid in the five normal individuals was greater than predicted (observed 16.25 ± 0.38 litres, predicted 15.81 ± 0.45 litres; P < 0.025).

Interstitial fluid was inversely correlated with blood pressure: diastolic r = −0.53, systolic r = −0.48 (P < 0.01 for both). The relationships of plasma volume and interstitial fluid to diastolic blood pressure, including all 30 subjects, are shown in Fig. 2.

Fig. 2. Plasma volume (■) and interstitial fluid volume (□) (as % predicted) against diastolic blood pressure (analysed in groups of 10 mmHg increments of blood pressure, means ± SEM), includes five normal and 25 hypertensive subjects (numbers in each group are given in parentheses); r = −0.30 for plasma volume and −0.53 for interstitial volume (P < 0.01 for both).

malities being corrected when a new steady state was achieved at the expense of an increased systemic blood pressure [16, 17], whereas if the relationship were initially normal then hypertension should result in sodium and water loss [15] with reductions in plasma and extracellular fluid volumes. Secondary renal changes may partially correct these losses as suggested by Safar et al. [33] who found that plasma volume reduction in hypertension was less than that expected from data relating plasma volume to blood pressure in normal subjects. Evidence supporting a secondary shift of the pressure–natriuresis curve in essential hypertension has recently been presented [34].

Early studies of plasma volume in hypertension produced inconsistent results [9, 19, 35–37]. However, these studies often considered hypertensive patients as a single homogeneous group as regards aetiology, presence of complications and concurrent medication and often failed to take into account the importance of posture [37], diet, diurnal variation [36] and vascular permeability [23] in the measurement of plasma volume. Moreover control groups were often not adequately matched and indices of reference for comparison not always appropriate.

In the present study the diagnostic category was strictly defined, complications were excluded, no patient had previously received antihypertensive or diuretic therapy, only one sex (males) was studied and the conditions under which the
tests were performed were standardized. Although sodium intake was not controlled sodium excretion was similar in the normal and hypertensive groups. The control group was well matched for height and body weight but did differ in mean age, although the ranges were similar. However, plasma volume, whether expressed as absolute values or when corrected for body build, was not influenced by age, an observation made by others [38, 39]. Extracellular fluid volume is also reported to be unaffected by age [31, 39]. In normal subjects plasma volume was significantly related to a number of body indices, but especially to body surface area as calculated from height and weight [32, 40]. Even so when expressed as a function of body surface area not all variability was eliminated; therefore plasma volume was expressed as a percentage of that predicted by body surface area from the regression analysis in normal subjects.

Plasma volume in men with essential hypertension was significantly lower than that predicted (Table 3), confirming other reports [20, 22, 41]. Also plasma volume was inversely correlated with both systolic and diastolic blood pressures [20, 22, 23]. The overlap between values in normal and hypertensive subjects underlines the arbitrary nature of this division. Packed cell volume was increased in hypertension and by assuming a normal peripheral/whole-body packed cell volume ratio in hypertension then erythrocyte volume was not similarly reduced (Table 3). This suggests a selective reduction in plasma volume rather than in all blood constituents. These results add to the evidence against plasma volume expansion being responsible for the blood pressure elevation in any phase of uncomplicated essential hypertension. Even when cardiac output is raised, as in labile or borderline hypertension [21, 42, 43], plasma volume is normal [44] or reduced [21]. In established essential hypertension plasma volume is reduced ([20, 22, 41], present study) which contrasts with expansion and positive correlation of plasma volume to blood pressure in renoprival hypertension [4].

Does this reduction in plasma volume reflect a uniform reduction in extravascular volumes in all tissues and organs, a selective loss from the extravascular compartment or an overall contraction of extracellular fluid? It has been suggested that cardiopulmonary blood volume may be functionally increased in essential hypertension even when total blood volume was reduced [45]. However, this is not supported by others [46] and the relevance of its relationship to cardiac output in hypertension is controversial. On present evidence a redistribution of blood or a functional increase in cardiopulmonary blood volume due to increased venous compliance is unlikely [47]. A disturbance of the partition of extracellular fluid between intravascular and interstitial compartments in essential hypertension has been proposed, as extracellular fluid volume has been reported as normal [6, 8–11] or expanded [12–14] in hypertension, whereas plasma volume was reduced. In studies in which plasma and extracellular fluid have been measured simultaneously the plasma/interstitial fluid volume ratio was decreased in hypertension [30, 41]. These results could be explained by increased vascular permeability or transcapillary leak [23, 48]. As well as accounting for decreased plasma volume they would also lead to methodological problems in determining plasma volume, which in most studies involved measurement of albumin space. Thus a single timed specimen could result in a lower value for plasma volume in hypertensive subjects. This error was avoided by taking several timed blood samples and calculating a value for the Evans blue concentration at zero time. The Evans blue clearance rate was only slightly and not significantly increased in hypertensive subjects. Although this is compatible with increased leakage of albumin other studies have shown that plasma proteins are normal or raised in hypertension [19]. Thus protein loss from the intravascular space is an unlikely cause of the reduced plasma volume.

Extracellular fluid volume was significantly reduced in the hypertensive patients studied and this difference could not all be attributed to the reduction in plasma volume (mean reduction 0.69 vs 0.23 litre respectively). A reduction in the extravascular component of the extracellular fluid was confirmed by finding a significant difference between observed and predicted interstitial fluid volumes. As the tables used for calculating the predicted extracellular fluid volume [31] gave an underestimate for the conditions of this study (in five normal subjects the observed value for interstitial fluid was significantly greater than that predicted) the interstitial fluid in essential hypertension was reduced by approximately 6% compared with 7% for plasma volume. Interstitial fluid volume was also inversely related to blood pressure and the relationship was similar to that for plasma volume and blood pressure (Fig. 2). The relationship between plasma volume and interstitial fluid is therefore preserved in essential hypertension, with a proportionate reduction in each.

These findings are analogous to data obtained in other forms of hypertension, e.g. phaeo-
chromocytoma [49], renovascular hypertension [4] and hypertension in pregnancy [50, 51] and some experimental models, e.g. spontaneously hypertensive rats [52] and two-kidney, one-clip hypertension in the rat [53]. In all these the ‘normal kidney’ is exposed to a raised perfusion pressure resulting in a pressure natriuresis and diuresis, and normal sodium balance is restored when the cause of the hypertension is corrected [54].

There is no evidence in the present study to support the hypothesis that in essential hypertension a primary pathogenetic mechanism is a resetting of the pressure–natriuresis curve which results in expansion of plasma and interstitial fluid volumes. In contrast both were found to be reduced with an inverse relationship to blood pressure. This contraction of extracellular fluid is most likely to reflect a natriuresis and diuresis secondary to the raised renal perfusion pressure.

Acknowledgment

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References

Volumes in hypertension


