24-h exchangeable sodium in genetically hypertensive and normotensive rats[1]

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Summary. Total exchangeable sodium (Na_e) was measured in NZ genetically hypertensive (GH) rats, random bred normotensives (N), Japanese spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats WKY. SHR rats were found to have significantly higher Na_e than any of the other 3 strains. This confirms results previously obtained using a different method for Na_e measurements.

We have previously found that the Japanese spontaneously hypertensive rat (SHR) has a higher total body sodium per kg body weight (Na_e) than either the normotensive Wistar-Kyoto (WKY) strain, the New Zealand genetically hypertensive (GH), or the random bred normotensive (N) strain from which the GH rat was originally bred[2,3]. The method used for measurement of total body sodium involved the rats being maintained on a sodium-free pellet diet with a choice of 0.5% NaCl (labelled with ^{22}Na) and distilled H_2O to drink. It was noted that the SHR had a greater salt appetite than the other 3 strains and therefore it was conceivable that their higher Na_e was an artefact due to their greater intake of NaCl. In an attempt to eliminate this possibility, it was decided to repeat the measurement in rats on a normal diet and water, using an isotope dilution method to measure 24-h exchangeable sodium.

Methods. 24-h exchangeable sodium. The method used was essentially as described by Gresson et al.[4]. Under light ether anesthesia the left jugular vein was exposed and 0.25 ml of NaCl solution containing 16 μCi/ml of ^{22}Na (Radiochemical Centre, Amersham) was injected slowly. Each rat was then weighed. 24 h later, the whole body radioactivity was counted in a whole-body gamma counter and the rats were then anesthetized with ether for determination of specific activity of ^{22}Na in body fluids. 2 ml of blood were withdrawn and placed in tubes containing lithium-based heparin for measurement of serum radioactivity and Na^+ concentration. The blood was centrifuged at 1000×g for 30 min and then placed in a water bath at 37 °C to allow clotting before the serum was pipetted off for analysis. Serum radioactivity (Packard gamma counter) and Na^+ concentration (flame photometer) were measured. A conversion factor was calculated by use of an external standard to allow for the difference in efficiency between the Packard and total body rat counter. The formula for calculation of total exchangeable sodium per kg body weight was as follows:

$$Na_e/kg = \frac{\text{total body radioactivity serum } Na^+}{\text{serum radioactivity}} \times \text{conversion factor} \times \frac{1000}{\text{wt}}$$

The body counter was the same as that used in the long-term Na_e studies referred to in the introduction[2,3].

Experimental groups. 1. Rats aged 10 weeks: GH, n=5; N, n=7; SHR, n=7; WKY, n=8.

2. Rats aged 16 weeks: GH, n=8; N, n=8; SHR, n=7; WKY, n=7.

All rats were bred in this Institute. The SHR and WKY rats were from breeders (SHR/N 44th generation, WKY/N 21st generation) supplied by Dr C.T. Hansen of the National Institutes of Health in 1978.

Statistical analysis. Analysis of variance was used to test the statistical significance of the differences between the group means. Means with standard deviations (SD) are given in all tables.

Results. Total exchangeable sodium. In the rats aged 10 weeks, 24-h Na_e was significantly higher in SHR than in GH ($p < 0.01$), N ($p < 0.01$), or WKY rats ($p < 0.05$). 24-h Na_e was significantly higher ($p < 0.01$) in WKY rats than in either GH or N rats but there was no difference in Na_e between GH and N rats (table 1).

In the rats aged 16 weeks, 24-h Na_e was significantly higher in SHR than in the GH, N or WKY rats ($p < 0.01$), and significantly higher in WKY rats than in GH rats ($p < 0.05$). There was no significant difference between the GH and N groups (table 1).

Discussion. Thus the finding of a significantly elevated Na_e in the SHR rats[2,3] has been confirmed by use of a 24-h method for measuring Na_e. The use of the 24-h method, in which the animals are maintained on a normal diet with tap water ad libitum, ensured that the SHR rats were not ingesting much larger amounts of NaCl than the other groups of rats.

As can be seen in table 2, where the Na_e results for the 24-h and long-term methods have been compared, the differences between the SHR and the other groups are of much the same order in both methods.

These results confirm the differences in Na_e between the SHR and other groups and suggest that there is a distinct difference in the pathophysiology of the hypertension of the SHR and GH. The present findings also serve to validate the use of the long-term method for measuring Na_e. This method has distinct advantages over the 24-h one, as it is possible to monitor Na_e over long periods under different experimental conditions, whereas the 24-h method is either terminal or at best can only be repeated after a reasonably long interval as it involves removal of up to 2 ml of blood for the necessary estimations.

Table 1. 24-h total exchangeable sodium (mmoles/kg)

	10 weeks		16 weeks	
	n	mean ± SD	n	mean ± SD
GH	5	41.28 ± 1.18**++	8	34.50 ± 1.27**+
N	7	41.67 ± 3.35**++	8	35.43 ± 0.77**
SHR	7	47.40 ± 1.57	7	38.46 ± 0.65
WKY	8	44.87 ± 1.38*	7	35.92 ± 0.77**

GH, genetically hypertensive; N, normotensive; SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto rats. *Significantly different from SHR, *$p<0.05$, **$p<0.01$; +significantly different from WKY, +$p<0.05$, ++$p<0.01$ (ANOVA).

Table 2. Na_e/kg. Percentage by which GH, N and WKY rats are lower than SHR. Comparison of Na_e by 2 methods

	24-h method		Long term method*	
	10 weeks	16 weeks	10 weeks	16 weeks
GH	12.9	10.3	9.0	8.5%
N	12.1	7.9	12.0	9.9%
WKY	5.3	6.6	4.7	9.4%

*Percentages calculated from values quoted in Simpson and Ledingham[3].

As far as we are aware, high Na_e in SHR rats has not been reported by others. The only report suggesting such a result was that published by Berglund et al.[5] in which a 7-day balance experiment indicated a greater sodium retention in SHR aged 16 weeks than in similarly aged WKY rats.

1 Acknowledgments. These studies were supported by the Medical Research Council of New Zealand.

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Hepatic oxygen consumption, in vivo, in the rat

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Summary. A method is described that quantitates hepatic oxygen consumption, in vivo, in the rat. This method can evaluate hepatic oxygen consumption resulting from chronic conditions that may alter it.

Hepatic oxygen consumption ($\dot{V}O_2$) is an important physiologic measurement whenever hepatic hemodynamic studies are performed. No method for, in vivo, $\dot{V}O_2$ has been evaluated in the rat. This report examines the feasibility for measuring, in vivo, $\dot{V}O_2$ and establishes the $\dot{V}O_2$ values in the rat.

Materials and methods. Male Sprague-Dawley rats (mean b. wt 326 g) were anesthetized with ketamine HCl (100 mg/kg b. wt i.m.). Polyethylene catheters (PE-50) were placed into the left ventricle and left femoral artery. The trachea was intubated with a soft plastic tube (2.8 mm OD) that was connected to a rodent respirator (Harvard Apparatus), set at a rate of 80/min and tidal volume adjusted for body weight. A 4-cm midline abdominal incision was made to the xyphoid and the animal was allowed to recover from the procedures.

Hepatic blood flows – hepatic arterial (HAF), portal venous (PBF) and total hepatic (THBF) – were measured by a left ventricular injection of ^{141}Ce-labeled microspheres (15 ± 3 µm diameter) with a reference sample[3, 4]. After the microsphere injection, the central liver lobe was rotated to the right (fig.). Two separate, 0.3 ml samples of hepatic venous blood were withdrawn into heparinized syringes that were capped with rubber luer lock tips and placed into iced water. Similar samples were obtained from the femoral artery catheter and from the portal vein, through a 21-gauge Butterfly needle, by rotating the duodenal loop to the left. The animal was sacrificed with saturated KCl.

Hepatic blood flows are calculated as previously described[3] and are expressed in $ml \cdot min^{-1} \cdot g\ liver^{-1}$. Arterial, portal venous and hepatic venous blood samples were measured for oxygen content by a total oxygen analyzer (Lex-O_2-Con)[5] and for blood gases (pH, pO_2, pCO_2) by a Corning blood gas analyzer (Model 165). Total hepatic oxygen delivery (O_2 delivery), $\dot{V}O_2$ and hepatic oxygen extraction (% extraction) were calculated from hepatic blood flows and oxygen content[6]. The results are shown as means ± SE and Student t-tests were used for statistical analysis.

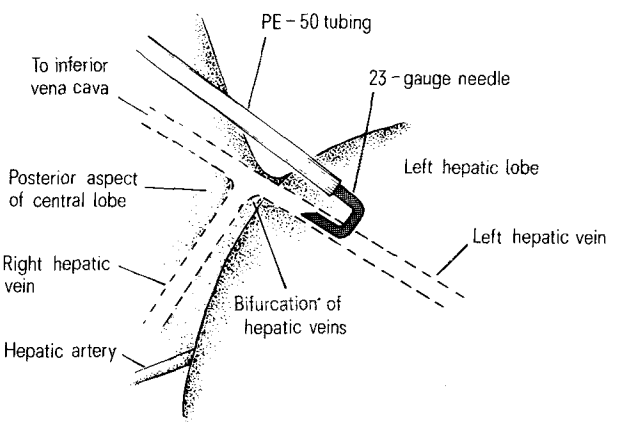
Results and discussion. Hepatic blood flows and $\dot{V}O_2$ were evaluated in 8 rats (table). Oxygen content (ml O_2/100 ml blood) was 15.7 ± 0.3 in arterial, 8.1 ± 0.5 in portal venous and 5.2 ± 0.3 in hepatic venous blood samples. Blood gas measurements (pH, pO_2, pCO_2) in arterial blood was 7.40 ± 0.02, 80 ± 4 mm Hg, 34 ± 2 mm Hg; in portal venous, 7.31 ± 0.01, 38 ± 1, 44 ± 2; and in hepatic venous, 7.37 ± 0.01, 26 ± 1, 41 ± 2.

We have also shown that mechanical ventilation does not affect hepatic blood flows since, in 13 nonventilated rats, HAF (0.35 ± 0.04), PBF (1.38 ± 0.11) and THBF (1.73 ± 0.11) were similar (p = NS) to the ventilated rats. Also, rotating the duodenal loop to expose the portal vein

Hepatic hemodynamics and oxygen consumption

	Mean ± SEM
HAF[a]	0.39 ± 0.05
PBF[a]	1.34 ± 0.10
THBF[a]	1.73 ± 0.09
O_2 delivery[b]	17.1 ± 0.9
$\dot{V}O_2$[b]	8.1 ± 0.8
% extraction	47.3 ± 2.9

[a] $ml \cdot min^{-1} \cdot g^{-1}$; [b] $ml\ O_2 \cdot min^{-1} \cdot 100\ g^{-1}$.



A preshaped, 23-gauge needle is inserted through the liver parenchyma into the left hepatic vein with the aspirating tip placed at the bifurcation of the left and right hepatic veins.