

# Nonuniform Distribution of Sodium in the Rat Hepatocyte

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**ABSTRACT** The volume of the nucleus, endoplasmic reticulum (including Golgi complex), mitochondria, and cytoplasmic ground substance was measured in rat hepatocytes by stereological methods. The Na content was also measured by flame photometry. Variations in Na content correlated significantly with variations in volume of nucleus and endoplasmic reticulum. From the correlation parameters, Na concentrations were estimated as follows: nucleus, 108 mM; endoplasmic reticulum (ER) (including Golgi complex) 27 mM; cytoplasm (including mitochondria and remaining organelles) 16 mM.

Evidence regarding the distribution of Na in cells is inconclusive. Measurements of intracellular Na activity have been recently reviewed (1). Zadunaisky (2) located Na in the transverse tubules of frog muscle by the pyroantimonate technique but the specificity of this has been disputed (3, 4). In toad oocytes, the Na activity coefficient measured by cation-selective glass microelectrodes is much lower than that in bulk solutions of the same composition (5); some Na is not replaceable by Li and autoradiography shows that this fraction lies in the cytoplasm and not the nucleus although poor resolution prevented more exact location (6, 7, 8). Electron microprobe analysis has been used to locate Na in a tissue such as frog skin (9) although it has so far proved difficult to obtain good intracellular localization.

The method of the present study was to correlate liver Na concentration determined by flame photometry with the volume of various subcellular components of the hepatocyte determined by stereological methods in both control and phenobarbitone-treated rats. By relating changes in Na concentration to changes in the volume of components, it is possible to make estimates of the Na concentration in each subcellular component. We have found that Na is not uniformly distributed in the hepatocyte and that Na concentration varies considerably from one subcellular component to another. This work has been briefly reported (10).

## MATERIALS AND METHODS

Eighteen Sprague-Dawley rats, weighing between 350 and 420 g, were used. Sixteen animals were paired as to age, weight, and sex; one of each pair was used as control and the other received phenobarbitone by injection (100 mg/kg daily for 4 days); there were two additional control animals (nos. 7 and 10). Laboratory diet and water were provided

ad libitum. Before sacrifice by ether inhalation on the 5th day animals were starved for 24 h to deplete liver glycogen. Three to five milliliters of blood was taken before exsanguination and removal and weighing of liver. For stereology blocks were fixed in buffered 4% glutaraldehyde, postfixed in Millonig's osmium fixative, dehydrated in changes of ethanol, and embedded in araldite. Silver sections were stained with uranyl acetate and lead citrate. Water content of fresh slices of the same liver was determined by reweighing after heating at 105°C for 24 h. Sodium in similar fresh liver slices and in plasma was determined with an EEL flame photometer (Evans Electro Selenium Ltd., London) after digestion overnight in 0.15 M HNO<sub>3</sub>. Density was measured by flotation in sucrose solutions and was found to lie between 1.05 and 1.07 g/cm<sup>3</sup>; a value of 1.06 g/cm<sup>3</sup> was used in calculations. Sampling of blocks and recording of micrographs was done by the methods of Weibel et al. (11), except that only magnifications × 2,500 and 10,000 were used. These were analyzed by means of a projection apparatus giving a secondary magnification of × 5.5 or 9 (12). At × 2,500 between 1,573 and 3,360 points were counted and at × 10,000 between 359 and 1655 points, and from these volume densities were calculated (12).

Extracellular Na was calculated from the stereologically determined extracellular space (control mean 0.103 cm<sup>3</sup>/cm<sup>2</sup> liver) assuming an Na concentration the same as that of the plasma; intracellular Na (control mean 21.4 mM) was calculated by subtraction from total Na. These values agree well with previous data of Williams and Woodbury (13) who found an extracellular space (1-h inulin space) of 9–12% and intracellular Na concentrations of 24.7 and 21.1 mmol/liter cell water in fasted male and female rats, respectively.

#### RESULTS

Basic data are shown in Table I. The only statistically significant effect of phenobarbitone was an increase in the specific weight of the liver and in the

TABLE I  
EFFECT OF PHENOBARBITONE ON LIVER

	Control, mean ± SD (n = 10)	Phenobarbitone, mean ± SD (n = 8)	Significance of difference*
Liver specific weight (g/100 g body weight)	2.52±0.22	3.24±0.47	P < 0.0005
Liver water concentration (g H <sub>2</sub> O/g fresh liver)	0.682±0.018	0.671±0.017	NS
Total liver Na concentration (μmol/cm <sup>3</sup> )	36.7±3.4	34.6±3.5	NS
Plasma Na concentration (μmol/cm <sup>3</sup> )	146.7±7.0	144.5±6.2	NS
Intracellular Na concentration (μmol/cm <sup>3</sup> )	21.5±5.4	21.4±4.8	NS
Volume density (cm <sup>3</sup> /cm <sup>3</sup> liver)			
Hepatocyte	0.882±0.041	0.896±0.027	NS
Nucleus	0.060±0.046	0.051±0.012	NS
Endoplasmic reticulum (including Golgi complex)	0.144±0.040	0.202±0.051	P < 0.01
Mitochondria	0.209±0.038	0.189±0.024	NS
Cytoplasmic ground substance (including remaining organelles)	0.472±0.037	0.457±0.054	NS
Littoral cells	0.014±0.008	0.013±0.005	NS
Extracellular space (including bile canaliculi)	0.103±0.034	0.092±0.027	NS

\* Calculated by one-tail *t* test.

TABLE II  
CORRELATION OF VOLUME DENSITY OF ORGANELLES WITH  
INTRACELLULAR Na

Animal no.	Intracellular Na $\mu\text{mol}/\text{cm}^3$	Volume density ( $\text{cm}^3/\text{cm}^3$ liver)			
		Endoplasmic reticulum	Nuclei	Mitochondria	Cytoplasmic ground substance
<b>Control</b>					
1	19.4	0.133	0.058	0.212	0.446
2	20.0	0.122	0.079	0.187	0.469
3	26.1	0.147	0.056	0.237	0.498
4	19.7	0.135	0.039	0.238	0.478
5	18.6	0.176	0.042	0.202	0.431
6	23.8	0.224	0.056	0.188	0.452
7	23.0	0.131	0.046	0.219	0.525
8	14.0	0.066	0.017	0.224	0.530
9*	33.4	0.146	0.182	0.124	0.472
10	17.4	0.161	0.028	0.260	0.423
<b>Phenobarbitone</b>					
1	23.5	0.181	0.056	0.170	0.495
2	22.2	0.157	0.046	0.178	0.489
3	21.2	0.230	0.067	0.233	0.383
4	23.5	0.286	0.054	0.162	0.429
5	28.8	0.244	0.064	0.214	0.403
6	16.7	0.218	0.041	0.177	0.469
8	22.1	0.131	0.032	0.190	0.549
9	12.9	0.170	0.049	0.189	0.442
Parameters of correlation with intracellular Na	<i>b</i>	+31.6	133.1	-11.0	-5.50
	<i>r</i>	+0.419	0.492	-0.074	-0.061
	<i>P</i> ‡	<0.05	<0.025	NS	NS

\* Values for control rat no. 9 were excluded from the initial correlations owing to the fact that both  $\text{Na}_i$  and  $V_m$  were exceptionally high. However, the predicted value of  $\text{Na}_i$  from Eq. 1 is  $33.3 \mu\text{mol}/\text{cm}^3$  which agrees with the observed value of  $33.4 \mu\text{mol}/\text{cm}^3$ .

‡ Calculated by one-tail test.

volume density of the endoplasmic reticulum (ER) (mainly in the smooth ER). However, when intracellular Na is correlated with the volume density of various subcellular components, grouping the control and phenobarbitone-treated animals together (Table II), significant positive correlations are found with volume of ER and nuclei. The correlations of intracellular Na with volume of mitochondria and cytoplasmic ground substance are negative and nonsignificant. Further analysis was done by calculating a multiple correlation between intracellular Na and volume of ER, volume of nuclei, and volume of cytoplasmic ground substance (including lysosomes and microbodies) and mitochondria added together. This resulted in the following equation:

$$\text{Na}_i = 27.0 V_{er} + 108.1 V_n + 16.3 V_{v(gs + mit)} \quad (1)$$

The significance of this multiple correlation is high ( $P < 0.001$ ), establishing that Na is not uniformly distributed in the hepatocyte. The regression coefficients may be interpreted as representing the concentrations of Na in ER (27 mM, SD 13), in nuclei (108 mM, SD 23), and in cytoplasm including mitochondria (16 mM, SD 3.6). The Na concentration in the nucleus is significantly greater than that in ER or cytoplasm ( $P < 0.0025$  and  $P < 0.0005$ ).

Two possible systematic errors with opposite effects may affect these values. First, ER volume may be underestimated due to Holmes' effect (12) leading to overestimation of Na concentration in it; on an extreme assumption of a 50% increase in ER volume (11) the Na concentration in ER falls to 22 mM while that in cytoplasm rises to 17 mM. Secondly, the method of regression analysis used assumes all random error to be in the Na concentration alone although random error also occurs in the volume densities; this may lead to considerable underestimation of Na concentration in ER and nucleus.

This interpretation does of course require the assumption that Na content is proportional to organelle volume and this might be questioned especially in relation to the high Na concentration in the nucleus which might involve adsorption of Na to some intranuclear component.

#### DISCUSSION

It is concluded that Na is not uniformly distributed in the hepatocyte. The high estimate for nuclear Na concentration is similar to the results of Langendorf et al. (14) who found a value of 131 mM. The low estimate for the Na concentration of cytoplasm and mitochondria suggests that the Na concentration of mitochondria is low, a suggestion in agreement with analytical data (15) from which a value of 6.8 mM may be calculated. However the present estimates of Na concentration are higher than those obtained by Uyeki (16), nucleus 30 mM, microsomes 7 mM, mitochondria 5 mM, cytoplasmic matrix 14 mM (approximate calculations using present values for volume density). Uyeki perfused the liver with sucrose solution before fractionation and analysis and this may have depleted the Na content.

The effect of phenobarbitone on the specific weight of the liver and on the volume density of the ER is in agreement with the results of Staubli et al. (17). It may be noted that although these authors found an increase in the specific volume of the nucleus there was no significant change in its volume density as in the present results. Phenobarbitone has in fact rather little effect on the volume densities of components other than the ER; its contribution to the present study consists in extending the range of ER volume densities to be correlated with Na concentrations.

Since its SD is high, the estimate of Na concentration in ER is not significantly different from that in cytoplasm ( $0.25 > P > 0.20$ ). Nevertheless, there is some suggestion that Na may be accumulated from the cytoplasm into the ER. In amoebae it is known that Na is concentrated in the contractile vacuoles before being expelled from the cell (18, 19). It is possible that a similar mechanism may occur in the ER of the hepatocyte.

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