Nonuniform Distribution of Sodium in the Rat Hepatocyte

G. HOOPER and D. A. T. DICK
From the Department of Anatomy, University of Dundee, Dundee, Scotland

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 Electroselenium Ltd., London) after digestion overnight in 0.15 M HNO₃. Density was measured by flotation in sucrose solutions and was found to lie between 1.05 and 1.07 g/cm³; a value of 1.06 g/cm³ 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 cm³/cm² 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 H₂O/g fresh liver) | 0.682±0.018 | 0.671±0.017 | NS |
| Total liver Na concentration (µmol/cm³) | 36.7±3.4 | 34.6±3.5 | NS |
| Plasma Na concentration (µmol/cm³) | 146.7±7.0 | 144.5±6.2 | NS |
| Intracellular Na concentration (µmol/cm³) | 21.5±5.4 | 21.4±4.8 | NS |
| Volume density (cm³/cm³ 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) | 0.105±0.054 | 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 \( \mu \text{mol/cm}^3 \) | Endoplasmic reticulum | Nuclei | Mitochondria | Cytoplasmic ground substance |
|------------|-----------------------------------------|-----------------------|--------|--------------|-----------------------------|
| **Control** |                                        |                       |        |              |                             |
| 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 0.258 0.478            |                       |        |              |                             |
| 5          | 18.6 0.176 0.042 0.202 0.451            |                       |        |              |                             |
| 6          | 23.8 0.224 0.056 0.188 0.452            |                       |        |              |                             |
| 7          | 23.0 0.151 0.046 0.219 0.525            |                       |        |              |                             |
| 8          | 14.0 0.066 0.017 0.224 0.530            |                       |        |              |                             |
| 9*         | 35.4 0.146 0.182 0.124 0.472            |                       |        |              |                             |
| 10         | 17.4 0.161 0.028 0.260 0.423            |                       |        |              |                             |
| **Phenobarbitone** |                                     |                       |        |              |                             |
| 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 0.181 0.032 0.190 0.549            |                       |        |              |                             |
| 9          | 12.9 0.170 0.049 0.189 0.442            |                       |        |              |                             |
| **Parameters of correlation with intracellular Na** | \( b \) +31.6 133.1 -11.0 -5.50 | \( r \) +0.419 0.492 -0.074 -0.061 | \( P \) <0.05 <0.025 NS NS |

* Values for control rat no. 9 were excluded from the initial correlations owing to the fact that both Na, and \( V_n \) were exceptionally high. However, the predicted value of Na, from Eq. 1 is 33.3 μmol/cm³ which agrees with the observed value of 33.4 μmol/cm³.

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:

\[
\text{Na}_t = 27.0 \, V_{er} + 108.1 \, V_n + 16.3 \, (V_{ps} + V_{mit}) \tag{1}
\]
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.
We wish to thank Mrs. E. M. Lloyd-Davies for her skilled assistance with electron microscopic techniques. This work was supported by a grant from the Medical Research Council.

Received for publication 14 October 1975.

REFERENCES

1. Lev, A. A., and W. Mcd. Armstrong. 1975. Ionic activities in cells. Curr. Top. Membranes Transp. 6:59-123.
2. Zadunaisky, J. A. 1966. The location of sodium in the transverse tubules of skeletal muscle. J. Cell Biol. 31:C11-C16.
3. Shin, S., V. Mizuhira, T. Amakawa, and Y. Futaesak. 1970. An analysis of the histochemical procedure for sodium ion detection. J. Histochem. Cytochem. 18:644-649.
4. Torack, R. M., and M. Lavalle. 1970. The specificity of the pyroantimonate technique to demonstrate sodium. J. Histochem. Cytochem. 18:635-645.
5. Dick, D. A. T., and S. G. A. McLaughlin. 1969. The activities and concentrations of sodium and potassium in toad oocytes. J. Physiol. (Lond.). 205:81-78.
6. Dick, D. A. T., and E. J. A. Lea. 1964. Na fluxes in single toad oocytes with special reference to the effect of external and internal Na concentration on Na efflux. J. Physiol. (Lond.). 174:55-90.
7. Dick, D. A. T., D. J. Fry, P. N. John, and A. W. Rogers. 1970. Location of inexchangeable sodium in the nucleus and cytoplasm of oocytes of Bufo bufo exposed to sodium-free solutions. J. Physiol. (Lond.). 210:305-319.
8. Dick, D. A. T., and D. J. Fry. 1975. Sodium fluxes in single amphibian oocytes: further studies and a new model. J. Physiol. (Lond.). 247:91-116.
9. Dorge, A., K. Gehring, W. Nagel, and K. Thurau. 1974. Localisation of sodium in frog skin by electron microprobe analysis. Naunyn-Schmiedebergs Arch. Pharmacol. 281:271-280.
10. Hooper, G., and D. A. T. Dick. 1975. Stereological determination of sodium distribution in the rat hepatocyte. Proceedings of the Anatomical Society. In press.
11. Weibel, E. R., W. Staubli, H. R. Gnagi, and F. A. Hess. 1969. Correlated morphometric and biochemical studies on the liver wall. I. Morphometric model, stereologic methods, and normal morphometric data for rat liver. J. Cell Biol. 42:68-91.
12. Weibel, E. R. 1973. Stereological techniques for electron microscopic morphometry. In Principles and Techniques of Electron Microscopy. M. A. Hayat, editor. Van Nostrand Reinhold Co., New York. 239-296.
13. Williams, J. A., and D. M. Woodbury. 1971. Determination of extracellular space and intracellular electrolytes in rat liver in vivo. J. Physiol. (Lond.). 212:85-99.
14. Lændorf, H., G. Siebert, I. Lorenz, R. Hannover, and R. Beyer. 1961. Kationenverteilung in Zellkern und Cytoplasma der Rattenleber. Biochem. Z. 335:273-284.
15. Borst, P. 1969. Biochemistry and function of mitochondria. In Handbook of Molecular Cytology. A. Lima-de-Faria, editor. North Holland, Amsterdam. 914-942.
16. Uyei, E. M. 1963. Intracellular distribution of cations and protein in regenerating rat liver. Am. J. Physiol. 204:257-261.
17. Staubli, W., R. Hess, and E. R. Weibel. 1969. Correlated morphometric and biochemical studies on the liver cell. II. Effects of phenobarbital on rat hepatocytes. J. Cell Biol. 42:92-112.

18. Chapman-Andresen, C., and D. A. T. Dick. 1962. Sodium and bromine fluxes in the amoeba Chaos chaos L. C. R. Trav. Lab. Carlsberg. 32:445-468.

19. Riddick, D. H. 1968. Contractile vacuole in the amoeba Pelomyxa carolinensis. Am. J. Physiol. 215:736-740.