Constancy of Intracellular Water as a Function of Dry Weight (36884)

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When reviewing the literature for data concerning the tissue uptake of various sugars and amino acids, one finds a wide divergence in the values assigned to the "extracellular space" which is usually expressed as ml of extracellular water/100 g wet weight of tissue. The reason for this variation becomes clear when one considers that different investigators do not remove excess water adhering to wet tissue in the same manner. Slight changes in blotting procedure and thus in total water content can create large differences in "extracellular space." This is obviously an unsatisfactory situation if one wishes to compare his data with that of others. However, the volume of intracellular water is independent of the blotting procedure providing that one does not exert undue pressure on the tissue and consequently inadvertently squeeze out intracellular water. The dry weight should also be largely independent of the blotting procedure. Therefore, a much more reasonable approach would be an expression of data based on these two constant values, intracellular water and dry weight. We propose that the use of an intracellular water to dry weight ratio (intracellular water/dry weight) would provide a more satisfactory means for the comparison of data among different laboratories. If one knows the value for this intracellular water/dry weight as well as the wet weight and the dry weight of the tissue, it becomes a simple procedure to calculate the "extracellular space" or, more importantly for uptake studies, the "intracellular space." It was our purpose to (1) blot tissues by different techniques in order to give rise to different "extracellular spaces" and (2) determine whether the intracellular water to dry weight ratio remains constant when "extracellular spaces" are significantly different.

TABLE I. Effect of Blotting on Extracellular and Total Water Contents and Intracellular to Dry Weight Ratio of Isolated Rat Atria.

No. of blottings	ml/100 g wet wt ± SE: Extracell water	ml/100 g wet wt ± SE: Total water	kg intracell water / kg dry wt ± SE
2	41.3 ± 3.1	79.7 ± 0.1	1.75 ± 0.14
20	32.4 ± 1.4	76.7 ± 0.4	1.91 ± 0.02
p	$<.05$	$<.001$	$>.1$

Methods. Atria were isolated from twelve 225–300 g male rats and were placed in 2 ml of modified Krebs Ringer bicarbonate glucose medium (1) containing 1.0 mM mannitol labeled with 0.5 μCi of mannitol-1-^{14}C. After one hour the atria were blotted either 2 (8 atria) or 20 (4 atria) times (2), weighed, dried to constant weight, and reweighed. These atria were then pulverized and digested for one hour in 0.5 ml Soluene-100 (Packard Instrument Co.), placed in 10 ml of Bray's scintillator solution (3) for subsequent counting with a Packard Tri-Carb Scintillation counter. An aliquot of medium was also taken for counting. From the amount of mannitol-1-^{14}C in the tissue and its concentration in the medium, the volume of distribution of this extracellular marker in the total tissue water (extracellular space) was determined. The total water volume minus the extracellular water was considered to be the intracellular water space.

Results. Values for the volume of extracellular water, the volume of total water, and the ratio of intracellular water to dry weight, were determined for tissues blotted by two methods. The atria blotted twenty times had 22% less extracellular and 4% less total water than did the atria which were blotted twice (Table I). The intracellular water to dry

TABLE II. Calculated Intracellular Water/Dry Weight Ratios from Data Obtained in the Literature.

Species	Tissue	ml/100 g wet wt: Extracell water	ml/100 g wet wt: Total water	kg intracell water / kg dry wt	Exposure to marker (hr)	Marker	Ref
Rat	Heart *in vivo*	11.2	75.3	2.6	6	Inulin	5
	Heart *in vivo*	24.9	78.5	2.5	15	Inulin	8
	Vent strip *in vivo*	20.6	77.2	2.5	3	Inulin	9
	Heart perfused	28.0	79.2	2.5	½	Inulin	7
	Heart perfused	30.5	80.0	2.5	½	Inulin	10
	Heart perfused	53.5	86.9	2.6	4	Inulin	6
	Heart perfused	32.4	81.2	2.6	1	Inulin	11
Cat	Papillary	18.1	75.3	2.3	1	Inulin	12
	Papillary	19.4	74.6	2.2	2	Inulin	12
	Papillary	21.1	75.4	2.2	3	Inulin	12
Guinea pig	Vent strip	26.4	78.0	2.3	3	Inulin	13
Rat	Vent strip	30.2	79.1	2.3	2	Inulin	9
	Heart perfused	38.0	80.0	2.1	½	Sorbitol	10
	Heart perfused	35.6	78.9	2.1	½	Sorbitol	14
Cat	Papillary	30.3	75.8	1.9	2	Mannitol	15

weight ratio, however, was independent of the blotting procedure.

Discussion. Atria which were blotted by two different techniques gave rise to different values for the calculated "extracellular space." In spite of the marked difference in the calculated extracellular water volume, the values for the intracellular water to dry weight ratio remained constant.

The concept of the constancy of the intracellular water to dry weight ratio was considered by Coret and Hughes (4). They cited the data of Dosekun and Mendel (5), Taylor *et al.* (6), and Bleehan and Fisher (7) in support of the constancy of this ratio despite wide variation in the extracellular water to dry weight ratios.

Several factors are known to affect the intracellular water to dry weight ratio. The nature of the extracellular marker used to define the intracellular space will affect this ratio (Table II). When inulin is used in the perfused rat heart, the ratio is 2.5–2.6. Sorbitol in the same tissue gave rise to a ratio of 2.1, suggesting that sorbitol gains access to tissue water which is not accessible to inulin. In a similar fashion, when mannitol and inulin were used as extracellular markers for cat papillary muscle, ratios of 1.9 and 2.2, respectively, were calculated.

The time allowed for equilibration to take place is also critical. The apparent intracellular space will be high before the extracellular marker has had time to equilibrate with the extracellular space. The ratio in the heart appears to be constant from the third to fifteenth hour with inulin as the marker *in vivo*. Perfused hearts also maintain a constant ratio with time between a 1/2 and 4 hr exposure to inulin.

Osmotic pressure is a third factor to consider when determining intracellular water to dry weight ratios. Page and Storm (16) have demonstrated a decrease in intracellular water space with increasing sucrose concentration to increase osmolarity.

Once the constancy of the intracellular water to dry weight ratio has been established for a tissue at a specific time period and osmotic pressure, it seems unnecessary to continue monitoring the extracellular space for each experiment as is commonly done with double label experiments in the same tissue measuring the cellular uptake of amino acids or sugar (17). Furthermore unless some variation in the experimental procedure can be shown to produce a change in the intracellular water to dry weight ratio it is probably better to use the same ratio in each experiment. Measurement of this ratio in each experiment is subject to the usual experimental errors and would result in the use of different ratios in each experiment where no real difference may exist. Also the technique of using one set of tissues for defining the extracellular space and another set for the uptake experiments (18) is subject to further error if variations in blotting exist in the two groups. The "extracellular space" and "intracellular space" can be calculated, if one knows the wet and dry weight (subtraction of which gives total water) of the tissue and the intracellular water to dry weight ratio constant for the experimental conditions.

We have shown that when tissues are handled in a manner which caused differences in the "extracellular space" values, intracellular water to dry weight ratio remained constant. This ratio can serve as a useful frame of reference for workers in different laboratories.

Summary. Rat atria were incubated for one hour in the presence of the extracellular marker mannitol. These atria were blotted either two times or twenty times resulting in markedly different values for the calculated "extracellular space." The ratios between the intracellular water and the dry weights were also determined for these same tissues and these ratios were not significantly different for the two blotting procedures. Therefore, although extracellular space values may vary, the intracellular water to dry weight ratio remains a constant, and thus a useful value under a specified set of conditions.

This investigation was supported by U.S. Public Health Service Grant HE 07718. We thank Dr. W. McD. Armstrong for stimulating discussions concerning this report.

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Received July 6, 1972. P.S.E.B.M., 1972, Vol. 141.