Organ and Whole Body Cell pH[1] (34743)

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The intracellular pH of various organs may be calculated from the transcellular distribution of the weak acid, 5,5-dimethyl-2,-4-oxazolidinedione (DMO) (1, 2). These calculations have been made for individual organs and for the whole body (3). Since in a heterogenous system of differing hydrogen ion activities, certain tissues may have a disproportionate effect upon the distribution of DMO (4), it is possible that whole body cell pH may not represent the mean or typical pH of the bulk of the tissues but rather primarily reflect the pH of one or two tissues which are quantitatively small but which might accumulate extraordinary quantities of DMO.

The value for intracellular concentration of DMO is obtained indirectly by subtraction of extracellular DMO from total DMO (1). Since these calculations utilize estimation of total body or tissue water and extracellular fluid volumes, it is clear that cell pH, whole body or tissue, which is calculated from the relative concentration ratios of DMO, must depend upon whatever markers are used to estimate space sizes.

In this study the distribution of DMO, water and extracellular fluid (chloride space) was measured in the organs of the rats and was compared with the distribution of these isotopes in the total body; corresponding cell pH values were calculated and compared. It was found that skeletal muscle cell pH is similar to and because of its bulk and buffering capacity, is probably the major controlling factor of whole body pH. Certain tissues such as testes, lung, intestines, skin, and skeleton have a calculated cell pH which is relatively high while other tissues, brain and spleen, have a relatively low cell pH. If sulfate rather than chloride space is utilized to estimate extracellular fluid volume, spleen and kidney cell pH is higher, skin and liver cell pH is lower, and skeletal muscle and whole body cell pH is about the same as calculated from chloride space.

Methods. Ten male albino rats, weighing 240–275 g, were injected intraperitoneally with either a mixture of 10 μCi of ^{3}HOH, 1 μCi of $Na^{36}Cl$ and 1 μCi ^{14}C-DMO in normal saline or saline alone. Seven rats were given the isotopic mixture; and the remaining three rats, who received only normal saline, acted as controls. All rats were then placed in metabolic cages and denied access to water and food. Three and one-half to 5.5 hr after injection the rats were decapitated in a guillotine and blood was immediately collected by inverting the severed body over a beaker coated with dried heparin. Subsurface blood was quickly drawn into a syringe for determination of pH and P_{CO_2}. The brain was then removed from the skull, and the heart, lungs, liver, kidneys, testes, spleen, and intestines (esophagus to anus including any contents and feces) were removed from the body. Any urine in the bladder was added to urine already collected in the metabolic cages. The animals were then skinned, leaving a carcass for separate analysis. Subsequent treatment of tissue, liquid scintillation counting, and calculation of pH was as described by Schloerb *et al.* (5).

The fraction of water in each tissue and plasma was determined by drying to constant weight in a vacuum oven at 40°.

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Organ content of isotope was calculated as follows:

$$\text{Isotope content} = \frac{5\,(^3H_p)\,(F_{t_{H_2O}})\,(\text{wt})}{(^3H_{dd})\,(Q_{3_H})\,(F_{p_{H_2O}})} \times (\text{isotope cpm})\,(Q_{\text{isotope}}),$$

where: 5 = dilution of tissue digest;
^3H_p = ^3H cpm/ml of plasma;
$F_{t_{H_2O}}$ = fraction of tissue which is H_2O;
wt = weight of tissue in g;
$^3H_{dd}$ = ^3H cpm/ml of diluted tissue digest;
Q_{3_H} = quench correction for ^3H in diluted tissue digest;
$F_{p_{H_2O}}$ = fraction of plasma which is H_2O;
isotope cpm = isotope cpm/ml in diluted tissue digest; and
Q_{isotope} = quench correction for isotope in diluted tissue digest.

Skeletal muscle isotope content was calculated by multiplying the muscle aliquot content by the amount of muscle (45.4% of body wt) found by Jackson and Lowrey in rats of similar type and weight (6). The remainder (carcass–skeletal muscle isotope content) was presumed to be mainly skeleton.

Results. Isotpe recovery was essentially complete: ^3H, 92.3 ± 11.6%; ^{14}C, 105.4 ± 6.3%; ^{36}Cl, 109.2 ± 10.6% (mean ± SD).

The distribution of organ weights and isotope contents is given in Table I. In comparison with ^{14}C-DMO there is considerable heterogeneity of the distribution of ^3HOH and ^{36}Cl. Skeletal muscle contains 52% of the body content of ^3HOH, 43% of the ^{14}C-DMO and only 27% of the ^{36}Cl. Skin, the next largest organ, has 17% of the ^3HOH, 22% of the ^{14}C-DMO and 30% of the ^{36}Cl. The only other sizable collection of isotypes is the intestines which range from 11 to 16% of the body contents of ^3HOH, ^{14}C-DMO, and ^{36}Cl. Fat contains very little isotope: 0.06% ^3H; 0.10% ^{14}C; and 0.14% ^{36}Cl of the body content per g of fat. The weight and method of calculation of the "remainder" suggest that this determination is mainly the skeleton (6). It is notable that the ^{36}Cl content of the "remainder" is quite high.

Mean blood pH was 7.40 ± 0.03 and P_{CO_2} was 37.5 ± 2.5 mm Hg (mean ± SD). Intracellular pH values of individual organs and of the whole body are listed in Table II. Again a heterogeneity is observed for calculations made with both chloride space and sulfate space. Utilizing chloride space, testes, lung, intestines, and the "remainder" have relatively high cell pH values as compared to

TABLE I. Distribution of Weight and Isotopes.

	Wt		^3HOH		^{14}C-DMO		^{36}Cl	
	%	±SD	%	±SD	%	±SD	%	±SD
Heart	0.39	0.00	0.53	0.00	0.58	0.00	0.49	0.08
Skeletal muscle	46.45	0.00	51.59	0.35	42.55	2.27	27.03	4.60
Testes	1.17	0.00	1.52	0.10	1.26	0.14	2.19	0.09
Brain	0.58	0.10	0.74	0.09	0.54	0.04	0.54	0.07
Spleen	0.29	0.04	0.34	0.00	0.26	0.00	0.36	0.00
Lung	0.51	0.06	0.66	0.04	0.76	0.08	1.15	0.12
Kidney	0.84	0.07	0.96	0.04	0.95	0.05	1.47	0.08
Intestines and contents	10.13	1.76	11.44	1.32	16.44	0.93	11.50	1.63
Skin	20.25	1.00	16.78	0.90	21.82	1.40	30.06	0.60
Liver	4.67	0.30	4.78	0.28	5.31	0.10	4.31	0.37
Removed for analysis	2.33	0.20	2.81	0.20	3.05	0.20	2.54	0.30
Remainder	12.39	1.34	7.88	1.42	6.48	3.56	18.36	6.83
Excreted (% of injected)			3.70	0.10	16.80	4.20	14.40	4.40

TABLE II. Intracellular pH.

Individual organs	Chloride space			Sulfate space[b]		
	pH	$(H^+)^a$	±SD	pH	$(H^+)^a$	±SD
Heart	6.83	147	70			
Skeletal muscle	6.82	151	33	6.80	159	31
Testes	7.17	68	14			
Brain	6.39	410	106			
Spleen	6.28	519	201	6.65	223	37
Lung	7.28	52	11			
Kidney	6.93	106	63	8.18	7	3
Intestines and contents	7.18	66	223			
Skin	7.03	93	127	6.33	370	240
Liver	6.99	103	33	6.32	385	268
Remainder	7.70	20	5	7.61	25	4
Whole body	6.81	155	41	6.89	129	26

[a] $M \times 10^{-9}$.

[b] Sulfate space calculated from data in Ref. (7) and (8).

whole body cell pH while brain and spleen have relatively low values. If sulfate space (7, 8) is equated with extracellular fluid volume instead of Cl, spleen and kidney cell pH values are higher and values for skin, liver, and "remainder" cell pH are lower.

Of major interest for this study is the similarity between skeletal muscle and whole body cell pH. Values for skeletal muscle cell pH utilizing both methods for estimation of extracellular volume and whole cell pH are practically indistinguishable; whole body cell pH calculated with the sulfate space of the "eviserated" rat (7) is slightly higher than these values.

Discussion. Despite heterogeneity in the distribution of organ isotope contents, skeletal muscle and whole body cell pH are similar. This similarity is also noted whether chloride or sulfate spaces are used to estimate extracellular fluid volume. The reasons for this similarity are: (i) the large proportion of the cellular mass which is skeletal muscle (6), (ii) the relative insensitivity of cell pH to errors or fluctuations in estimation of extracellular fluid volume (1), and (iii) the fact that there are not large disproportionate collections of DMO in any organ (Table I). Though this relationship is true for the normal rat (3) (Table II), it may not necessarily hold under other experimental conditions. However, it has been shown in the dog that skeletal muscle and whole body cell pH are closely related in the normal (2, 3, 9) and under conditions of potassium deficiency associated with both low and normal chloride stores (9) and in man under various conditions (3).

The cell pH values reported here for other organs must be evaluated further before they are accepted. DMO is transported against a concentration gradient in the hamster small intestine (10), and perhaps elsewhere. In organs which have large collections of transcellular fluids which may have a pH vlaue different from extracellular pH, there is the possibility of large fluxes of DMO due to nonionic diffusion (11) as well as active transport of chloride (12, 13).

Though DMO is lipid soluble, it (and other isotopes) do not accumulate to any significant extent in fat; with a large assumed value of 15% of body weight as fat only about 4% of body DMO would be found in the fat.

The question of the proper value to use for the extracellular volume of the whole body and of various organs is still unsettled. However, whole body cell pH is similar in the rat when chloride or sulfate spaces are used (Table II) and in the dog when chloride, sulfate or inulin spaces are used (9).

Summary. Intracellular pH values of 11 organs of the rat were compared with whole

body cell pH calculated from the distribution of tritiated water, ^{14}C-DMO, and ^{36}Cl. Heart and skeletal muscle cell pH were similar to whole body cell pH; testes, lung, kidney, intestines, skin, liver, and skeleton were higher while brain and spleen were lower. When sulfate rather than chloride spaces were used as an estimate of extracellular fluid volume, skeletal muscle pH was little changed but spleen and kidney were higher and skin and liver were lower.

The close approximation of skeletal muscle and whole body cell pH is due to the large fraction of isotopes present in muscle and the lack of significant or large disproportionate collection of isotopes elsewhere.

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