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Evaluation of Wet and Dry Weight Bases for Expression of Respiratory Rate *in vitro*.*

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Much of the recorded data on tissue respiration *in vitro* have been calculated on a dry, rather than a wet, weight basis on the assumptions that dry weighings are more accurate and that dry weight, multiplied by 5, gives the wet weight with sufficient accuracy.[1] Because of the fundamental consequences of these assumptions, we have undertaken to determine their validity.

Thirty-three virgin, post-pubertal albino rats, 15 males and 18 females, belonging to 7 litters of Slonaker-Wistar stock, were used. Age range was 98 to 120 days, mean age 107.7 days, coefficient of variation of age, 6.09%. This limited range was chosen to minimize the decrease with age of oxygen consumption[2-5] and of tissue water content,[6] reported for this and other species. Tissue respiration was determined by the Warburg method,[1] with the several precautions suggested by Dixon,[7] in a water bath at 37.5°±0.01°C. In every instance (317 cases) both wet and dry weight as well as oxygen consumption were ascertained.

Comparison of the variability of respiratory rate calculated on the wet and dry weight bases. The symbol Q_{O_2}, denoting oxygen consumption in ml, N.T.P., per g per hour, is commonly used to express respiratory rate *in vitro*. If the difference in accuracy of wet and dry weighings is critical, the variability of Q_{O_2} would be greater when computed on the basis of the less accurate weighing, other things being equal. To determine whether this were so, the coefficients of variation of Q_{O_2} were calculated for the organs and tissues which contribute most, in a quantitative sense, to the resting

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1 Warburg, O., 1926, *Uber den Stoffwechsel der Tumoren*, Berlin.

2 Boothby, W. M., Berkson, J., and Dunn, H. L., *Am. J. Physiol.*, 1936, **116**, 468.

3 Davis, J. E., *Am. J. Physiol.*, 1937, **119**, 28.

4 Pearce, J. M., *Am. J. Physiol.*, 1936, **114**, 253.

5 Field, J., 2d, Tainter, E. G., Martin, A. W., and Belding, H. S., *Am. J. Ophthal.*, 1937, **20**, 779.

6 Adolph, E. F., *Physiol. Rev.*, 1933, **13**, 336.

7 Dixon, M., 1934, *Manometric Methods*, Cambridge.

TABLE I.

Group numbers denote order of descending value of mean wet/dry ratio.
Column 1. No. of cases.
Column 2. Mean value of wet/dry wt ratio.
Column 3. Standard error of mean wet/dry wt ratios.
Column 4. Coefficient of variation of mean wet/dry wt ratios.
Column 5. Coefficient of variation of Qo_2 on wet wt basis.
Column 6. Coefficient of variation of Qo_2 on dry wt basis.

Group No.	Organ Preparation	1	2	3	4	5	6
V.	Skeletal Muscle:				%	%	%
V.	Diaphragm	90	5.08	.085	15.9	18.2	22.2
V.	Abdom. obliques	26	5.26	.139	13.5	20.5	26.2
V.	Semimembranosus	20	5.39	.213	16.8	15.6	16.1
V.	Trapezius	17	4.90	.252	21.3	27.6	16.8
IV.	Intestinal Wall	21	5.60	.263	21.5	25.4	32.9
IV.	Heart Muscle	10	5.61	.262	14.8	23.0	13.7
III.	Kidney	26	6.03	.156	13.2	11.6	13.6
V.	Liver	24	5.07	.102	9.9	18.7	14.2
I.	Spleen	6	7.74	.550	17.4	11.0	10.5
II.	Lung	6	6.64	.264	9.7	7.3	9.0
	Cerebrum:						
II.	Cortex	12	6.53	.228	12.1	9.4	11.9
V.	Medulla	11	5.07	.204	13.4	17.4	22.3
VI.	Skin	15	2.54	.083	12.7	42.1	41.4
VII.	Bone:						
VII.	Femur	10	1.58	.048	9.6	22.6	27.5
	Rib	10	1.44	.047	10.1	48.2	45.7
	Cranium	6	1.39	.066	11.7	—	—
VI.	Cartilage	7	2.44	.285	30.9	34.2	29.1

metabolism. The several values so obtained are given in columns 5 and 6, Table I.

It was found by statistical analysis that the differences between the coefficients of variation of Qo_2 calculated on the wet weight basis (column 5) and the dry weight basis (column 6) would occur at least 9 times out of 10 in random sampling of a series in which no real difference existed.[8] Hence there is no significant difference in variability of Qo_2 on these 2 bases. An important theoretical consequence is that tissue water content is related as closely, in a statistical sense, to the factors determining Qo_2 as is the miscellaneous aggregate of substances making up the dry weight. Since comparison of respiratory rate *in vitro* is more readily made on the basis of wet weight, and since the variability of tissue Qo_2 is no greater on this basis, expression of Qo_2 as a function of wet weight will often be the procedure of choice. It seems probable that similar reasoning could be applied to other metabolic measurements *in vitro,* such as aerobic and anaerobic glycolysis.

[8] Fisher, R. A., 1934, *Statistical Methods for Research Workers,* London, pp. 117-120.

Wet-dry weight ratios (W/D ratios). The number of cases, mean value, standard error and coefficient of variation of the W/D ratio for the several organs and tissues are given in the first 4 columns of Table I. From inspection of these data it can be seen that the assumption that W/D = 5 holds quite well for skeletal muscle, cerebral medulla and liver, but not for the other organs and tissues. The significance of the differences in mean W/D ratios is shown in Table II. Here the several organs and tissues have been arranged in 7 groups in order of descending values of mean W/D ratio. An arbitrary scale of 0 to 4 (*c.f.*, legend, Table II) has been used to indicate the reliability of the differences observed.

The chance of random occurrence of the differences in mean W/D ratio between adjacent groups is always 1 or less in 10, while the differences between groups separated by an intervening group would occur by chance only 1 or less times in 100. It seems reasonable to regard these latter differences (denoted by 2 in Table II) and greater ones (denoted by 3 or 4) as due to other than chance variation. Therefore, under the conditions of our experiments, no single value can be assigned for the W/D ratio of the several organs and tissues of the post-pubertal albino rat.

TABLE II.

Evaluation of significance of differences in mean wet/dry wt ratio. The symbols 0 to 4 indicate, in increasing significance, the probability that the observed differences could not occur in random sampling.

Quantitatively these symbols have the following meaning:

0. Difference might occur by chance oftener than 1.5 times in 10
1. " " " " " 1 or less times in 10
2. " " " " " 1 " " " " 100
3. " " " " " 1 " " " " 1000
4. " " " " " 1 " " " " 10000

	Bone VII	Cartilage VI	Skin VI	Liver V	Muscle V	Cerebral Medulla V	Heart IV	Intestine IV	Kidney III	Cerebral Cortex II	Lung II
I Spleen	4	4	4	4	4	4	3	3	2	1	1
II Lung	4	4	4	4	4	4	2	2	1	0	
II Cerebral cortex	4	4	4	4	4	3	2	2	1		
III Kidney	4	4	4	4	4	3	1	1			
IV Intestine	4	4	4	1	1	1	0				
IV Heart	4	4	4	1	1	1					
V Cerebral medulla	4	4	4	0	0						
V Skeletal muscle	4	4	4	0							
V Liver	4	4	4								
VI Skin	4	0									
VI Cartilage	3										

Summary. It has been shown in the case of the post-pubertal albino rat that: 1. The variability of Qo_2, based upon wet weight determinations, is no greater than when based upon determination of dry weight. 2. Organs and tissues differ significantly in respect to mean value of wet/dry weight ratio.

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Effect of Thyroxine on Eruption of Teeth in Newborn Rats.

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The relation of the thyroid gland to the rate of differentiation of the organism is well known. An excess of the thyroid hormone causes the individual more rapidly to assume its adult structure, as, for instance, in the effect of thyroxine in the acceleration of metamorphosis in tadpoles, or in the more rapid appearance and uniting of ossification centers in the bones of various mammals. Conversely, a thyroid hormone deficiency decreases growth and differentiation and the organism tends to remain infantile. Although this tendency is well recognized and accepted, a more thorough study as to the effect of thyroxine on the rate of differentiation of the various organ systems of the mammal is indicated.

Hoskins[1] first began this type of study in young rats. They were injected starting at the third day after delivery with 0.1 mg of acetyl-thyroxine at 2- to 3-day intervals until the fifteenth day. She observed a more mature external appearance, the fur appeared more rapidly, the nails were longer, the shape of the skull narrower and epiphyseal activity was increased.

In the preliminary stages of a more detailed investigation of a similar problem we noted that the injection of thyroxine had a very marked and specific effect on the rate of eruption of teeth that was more noticeable than any other single change in the young rat. Since Hoskins began her injections on the third day and spaced them at 2- to 3-day intervals her quantitative observations were not as accurate as ours, but she states "Another difference to be seen at this period is the precocious eruption of the incisor teeth, which are visible at least 2 days earlier in the injected than in the control ani-

[1] Hoskins, M. M., *J. Exp. Zool.*, 1927, **48**, 373.