Comparison of Respiratory Enzyme Levels in Tissues of Mammals of Different Sizes. (20169)

GEORGE H. FRIED* AND SAMUEL R. TIPTON. (Introduced by D. Frank Holtman.)

From the Department of Zoology and Entomology, The University of Tennessee, Knoxville.†

Various structural and functional features of an organism are known to vary with body size(1). The decrease in respiratory metabolic intensity with increase in body size is reflected in a decrease of the respiratory intensity of several homologous tissues(2).

There is then an inverse correlation of both total and tissue metabolic intensity and body size among different species, although intraspecific comparisons do not always demonstrate this relationship(3). Since there appear to be differences in the *in vitro* respiration of the homologous tissues of mammals of different sizes, it would appear that the next logical step would involve analysis of the intrinsic factors involved in cellular respiration to determine whether they too will demonstrate an inverse relationship with body size. Therefore succinoxidase and malic dehydrogenase activity in the tissues of the mature cow, rat, and mouse was measured because these enzymes are involved in the major metabolic pathways of the cell.

Methods. Albino rats and mice were sacrificed by decapitation after one day of fasting and all tissues were homogenized in glass tubes (4) with glass pestles turned at 1700 R.P.M. Malic dehydrogenase was determined by the method of Potter(5). Manometer readings were taken for two 5-minute periods, following a 6-minute equilibration with a last check reading taken for a final 10-minute period. The second 5-minute period was selected for the calculation since it gave the most reproducible results. Enzymatic assays are expressed as Q_{O_2} (mm^3 oxygen/mg dry wt/hr). Succinoxidase was determined by the method of Schneider and Potter(6) which essentially is a measure of succinic dehydrogenase activity, since cytochrome oxidase is in excess in the tissues studied. After a 10-minute equilibration, readings were taken for four 10-minute periods. Enzyme assays were made on the basis of the second 10-minute period.

Results. As the data presented in Table I indicate, with the exception of liver malic dehydrogenase of mouse and rat, the activities of the tissue enzymes decrease with increase in body size, but the enzyme activity-body size ratios show considerable quantitative variation. There is no significant difference in the activity of tissue enzymes from animals of the same species even with considerable variation in body weight, as shown in Table II.

Discussion. Although the data in Table I show that the decrease in enzyme activity with body size is very significant, the lack of a logarithmic relationship suggests that con-

TABLE I. Enzyme Q_{O_2} Values for the Tissues of Cow, Rat, and Mouse.

Animal	No. of exp.	Wt, kg	Succinoxidase: Liver Q_{O_2}	Heart Q_{O_2}	Kidney Q_{O_2}	Brain Q_{O_2}
Cow	11	340	21.1 ± 4.0*	51.8 ± 7.8	69.5 ± 5.0	20.9 ± 2.0
Rat	14	.205	60.1 ± 2.8	187.0 ± 11.0	103.0 ± 5.0	42.0 ± 2.3
Mouse	12	.031	102.0 ± 4.0	221.0 ± 14.0	189.0 ± 8.0	60.5 ± 2.1
			Malic dehydrogenase:			
Cow	11	340	64.2 ± 19.1	51.6 ± 24.1	53.3 ± 13.2	20.4 ± 2.8
Rat	11	.230	104.0 ± 12.0	132.0 ± 13.0	75.0 ± 7.0	26.7 ± 3.4
Mouse	10	.030	105.0 ± 21.0	170.0 ± 54.0	91.8 ± 9.7	57.7 ± 2.0

* Stand. dev. $= \frac{Sd^2}{n-1}$.

* Partial fulfillment of requirements for the Doctor of Philosophy degree at The University of Tennessee.

† Contribution No. 85 from the Department of Zoology and Entomology of the University of Tennessee.

TABLE II. Some Intra-specific Differences in the Enzymatic Activities in Mice and Rats.

Wt, g	Kidney Q_{O_2}	Heart Q_{O_2}	Liver Q_{O_2}	Brain Q_{O_2}
	Succinoxidase—Mice			
16	153	188	115	72.4
26	168	203	116	54.2
32	162	208	115	67.5
38	218	234	84.2	57.3
46	160	240	101	55.5
	Malic dehydrogenase—Rat			
230	68.4	127	121	
360	96.0	139	96.9	
	Succinoxidase—Rat			
140	86.5		52.0	40.0
172	95.7		59.6	29.9
210	108		62.5	49.5
265	93.0		54.7	34.1

trolling factors may be different from those effective in basal metabolism(1), cytochrome c(7), and cytochrome oxidase(8). The constant enzymic activity within a single species indicates indirect genetic factors regulating metabolic enzymes rather than hormonal or other direct somatic factors. That the enzymatic variation might be due partly to differences in homogenization, to deleterious effects of freezing (cow), or to dietary differences in the different species appears improbable under the experimental conditions.

Summary. Heart, kidney, liver, and brain of the cow, rat, and mouse were assayed for maximal activity of succinoxidase and malic dehydrogenase. The levels of the two enzymes generally reflect the inverse relationship of metabolism with body size in that the tissues of the cow were lowest, those of the rat intermediate, and those of mouse highest in activity for these enzymes. The enzyme levels did not vary as an exact power function of body weight, nor were there any intraspecific changes in enzyme levels with changes in body size.

1. Adolph, E. F., *Science*, 1949, v109, 579.
2. Krebs, H. A., *Biochimica et Biophysica Acta*, 1950, v4, 249.
3. Bertalanffy, L. von, and Pirozynski, W. J., *Science*, 1951, v113, 599.
4. Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, v114, 495.
5. Potter, V. R., *J. Biol. Chem.*, 1946, v165, 311.
6. Schneider, W. C., and Potter, V. R., *J. Biol. Chem.*, 1943, v149, 217.
7. Rosenthal, O., and Drabkin, D. L., *J. Biol. Chem.*, 1943, v150, 131.
8. Kunkel, H. O., and Campbell, J. E., Jr., *J. Biol. Chem.*, 1952, v198, 229.

Received February 17, 1953. P.S.E.B.M., 1953, v82.

Hemolytic Action of Water Soluble Compounds Related to Mephenesin. (20170)

F. M. BERGER, C. V. HUBBARD, AND B. J. LUDWIG.
From Wallace Laboratories, Inc., New Brunswick, N. J.

The description of the muscle-relaxing and paralyzing action of mephenesin(1) led to extensive trials of the drug as a substitute for curare during anesthesia(2). The use of concentrated solutions of mephenesin by the intravenous route was, however, soon abandoned because of the frequent occurrence of intravascular hemolysis, hematuria and anuria (3). During the past few years a number of compounds structurally related to mephenesin have been discovered that have higher water solubility than mephenesin and because of this appear more suitable for intravenous administration. The present paper describes the hemolytic action of several of these compounds and compares this property with the paralyzing and lethal action of these drugs.

Methods. Determination of water solubility was made by spectrophotometric measurement of saturated solutions of each of the compounds at the wave length corresponding to maximum absorption, using the specific absorbency previously determined for each of the compounds. The partition ratio of the compounds between water and oil ($K_{w/o}$) was likewise measured spectrophotometrically using cottonseed oil, U.S.P. as the oil phase. The partition ratio of the non-aromatic ether No. 13 was obtained by measurement of the refractive index of the aqueous solutions.