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Different Expressions for Enzyme Activities in Organs of Rat. Application to Aspartate Transaminase, Glutamate Dehydrogenase and AMP-Deaminase *

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The widely used activity expressions for enzyme levels in tissues are discussed: microkatals per unit of tissue weight, protein weight, and DNA weight. The expression of microkatals present in a definite organ in reference to a standard animal weight, 100 g in the case of rat, is also used. The different expressions are applied to aspartate transaminase, glutamate dehydrogenase and AMP deaminase activities in liver, hind leg striated muscle and kidneys in rat. The conclusion is reached that measurements of enzyme activity in tissues should be expressed in more than one form, as the information drawn from one could differ substantially from that obtained from other, giving artifactual views of the metabolic role played by the enzyme in a given tissue.

There has been considerable work done on the standardization of enzyme activity units (4, 5, 7, 8), but little has been done in relation with specific activity expressions. Probably one of the most adequate expressions of enzyme activity would be the indication of their molar concentration in any given tissue, as postulated by SRERE (15, 16), but the problems that this type of expression implies are considerable, because the molecular weight of the enzyme must be known, as well as the real space in which the enzyme develops its activity (i.e. mitochondrial space, etc.) (15), together with the problem of fitting the concept of «molarity» to enzymes

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attached to membranes or other fixed structures.

The most commonly used enzyme specific activity expressions are those that relate the enzyme activity to a given weight of sample (wet or dry) or to its protein content, the last being employed mainly when supernatants of homogenates are used, probably because there is no direct relation between the enzyme concentration in the homogenate and the size of the sample taken. It is less used the expression per cell unit (or DNA content), mainly restricted to research done with tissue cultures or Microbiology, but not widely used in investigations with animal organs and tissues. This paper deals with the complementary use of several expressions of the results, with the aim of determining the relative importance of a given enzyme activity in a tissue or organ, and also for the animal considered as a single unit.

Materials and Methods

Virgin female Wistar rats weighing 180.1 ± 7.8 g were used. The animals were fed rat chow *ad libitum* in a temperature controlled (23 ± 1° C) environment with a light cycle of 12 hours on and 12 hours off. The animals were killed by decapitation at the beginning of the light cycle. Pieces of liver, kidney and hind leg muscle were quickly dissected, weighed and homogenized in 10 volumes of chilled medium [KREBS-RINGER bicarbonate buffer (3)], containing 0.1 % dextran (Sigma, average molecular weight 200,000), 0.05 % bovine serum albumin (Sigma), 5 mM 2-mercaptoethanol and 0.1 % Triton X-100 (Rohm and Haas), using a teflon-glass motor driven Potter-Elvejhem type homogenizer. Crude homogenates were coarsely filtered through cheesecloth, and were kept in ice until analysed.

Aspartate aminotransferase (L-aspartate: 2-oxoglutarate aminotransferase, E.C. 2.6.1.1) and glutamate dehydrogenase (L-glutamate: NAD(P) + oxido-reductase — deaminating —, E.C. 1.4.1.3) were measured in the crude homogenates with spectrophotometric methods (1, 14) at 25° C using NADH as coenzyme. AMP-deaminase (5′-adenylate aminohydrolase, E.C. 3.5.4.6) was measured at 37° C with the procedure of MAKAREWICZ and STANKIEWICZ (9, 11, 12, 17), using AMP (Sigma) as substrate, and detecting the ammonia formed with the BERTHELOT indophenol procedure (2, 6). In this case, as the reaction had to be stopped discontinually, aliquots of the reaction mixture plus crude enzyme preparation were extracted at 0, 8 and 16 minutes, and the reaction was stopped with trichloroacetic acid, at a final concentration of 7.5 % (w/v) for posterior color development with the phenol nitroprussiate reagents. In all cases, the reaction initial velocities were determined by extrapolating the changes found at given times, and then were converted into katals and expressed as specific activities. Protein concentration was determined in the filtered crude homogenates by means of the FOLIN phenol procedure (10, 18), using bovine serum albumin (Sigma) as standard. The same source was used for estimation of DNA with the use of ethydium bromide (13), after ribonuclease treatment of the samples. Total muscle mass was estimated with the method of AROLA, HERRERA and ALEMANY (unpublished), based on the quantification of a specific muscle fraction in a sample of hind leg muscle and in the whole carcass. The fraction selected was the 1 M LiCl-soluble, distilled water-insoluble fraction of structural muscle proteins.

Results and Discussion

Table I summarizes the DNA and protein content of liver, kidney and hind leg striated muscle, as well as their mass percent in relation to the body weight of the animals used.

Table I. *Relative weight, protein and DNA content of the tissues and organs used in the experiments.*
All data are mean ± s.e.m. of 7 animals.

Organ	Organ weight expressed as % b.w.	Content in the homogenate (g/100 g tissue) Protein	DNA
Liver	4.06±0.15	16.3±0.8	0.57±0.07
Striated muscle	44.1±2.7	11.5±1.3	0.12±0.01
Kidneys	0.79±0.02	12.9±0.8	0.34±0.02

In table II the specific activities of aspartate aminotransferase, glutamate dehydrogenase and AMP deaminase of these three tissues have been displayed with reference to four types of units. The expression of enzymatic activity per unit of tissue weight is probably the most used unit of enzymatic specific activity, and it is probably due to its ready accessibility and meaning. The reference to protein crude extract content, however, is a further attempt in the unification of data, as it emphasizes the relative importance of the enzyme in the context of cell protein, while the expression with regard to DNA indicates the relative amount of the enzyme present in the cell.

Another expression shown in table II refers to the amount of enzyme activity found in the whole organ, corrected according to the weight of the animal. This is a more complex approach, as it refers to the whole animal's body taken as a single functional unit, thus the relative weight of the different tissues studied is taken into account to ponderate the relative importance of the different tissue enzyme activities, considered in the general context of the organism studied.

The three enzymes studied in this paper represent examples of enzymes unevenly distributed when expressed in activity per tissue weight unities. Thus, aspartate transaminase shows similar specific activities in the different tissues studied, while glutamate dehydrogenase is more concentrated in liver and kidney than in muscle, and AMP deaminase follows a reversed pattern to that of glutamate dehydrogenase, being its activity maximal in muscle. When expressed per unit of tissue weight, the values for aspartate transaminase indi-

Table II. *Aspartate ar ;inotransferase, glutamate dehydrogenase and AMP-deaminase specific activities in the liver, striated muscle and kidneys of female virgin rats expressed in four different ways.*
All data are mean ± s.e.m. of 7 different animals.

Enzyme/orqan	μkat/100 g of tissue	μkat/g of protein	μkat/g of DNA	μkat/100 g * of animal weight
Aspartate aminotransferase				
Liver	166 ± 11	10.3 ± 0.7	289 ± 29	6.89 ± 0.53
Striated muscle	106 ± 7	10.2 ± 1.0	904 ± 79	46.9 ± 3.2
Kidneys	137 ± 9	10.8 ± 0.7	408 ± 26	1.08 ± 0.07
Glutamate dehydrogenase				
Liver	209 ± 20	12.90 ± 1.24	374 ± 42	8.75 ± 1.02
Striated muscle	3.5 ± 0.5	0.31 ± 0.04	29 ± 5	1.55 ± 0.25
Kidneys	92.9 ± 6.3	7.24 ± 0.42	279 ± 25	0.73 ± 0.05
AMP deaminase				
Liver	5.9 ± 0.8	0.37 ± 0.05	10.5 ± 1.5	0.25 ± 0.05
Striated muscle	389 ± 72	34.7 ± 5.6	3140 ± 499	171 ± 32
Kidneys	14.5 ± 2.3	1.18 ± 0.22	43.0 ± 7.0	0.12 ± 0.02

* μkat in the whole organ corrected to an uniform animal size of 100 g.

cate a relative higher activity of the enzyme in the liver than in the kidney or muscle. The data are, however, more equalized when expressed per unit of protein weight, and the initial pattern is reversed when the data are expressed per unit of DNA weight (table II), as a consequence of the lower size of liver cells related to those of kidneys and the big ones of muscle. This relative abundance of aspartate transaminase in muscle is more emphasized when the data are corrected for relative weight of the organs studied, due to the considerable mass of muscle as related with the other organs.

The situation is not the same with glutamate dehydrogenase, as here there is already a considerable difference in the values expressed per unit of tissue weight, with muscle activity about one sixtieth of the liver one, being the activity of the kidney about one half of that found in the liver. The pattern is not sensibly changed when the data are expressed per unit of either protein or DNA weight. However, when the data are referred to unit of animal weight all muscle enzyme now accounts for about 18 % of the liver activity, whereas kidney enzyme dropped to a mere 8 %.

The pattern of AMP deaminase distribution in the organs of the rat follow a reversed pattern in relation to that of glutamate dehydrogenase, as the activity in muscle is about 66 times higher than that of liver when expressed per unit of tissue weight, 94 times higher when expressed per weight of protein, about 300 when expressed per unit of DNA weight and a staggering 686 times higher figure when corrected for the tissue weight. The activity of AMP deaminase in kidney is about 2.5 times higher than in liver when expressed per unit of tissue weight, being 3.2 and 4.1 times higher when expressed per unit of protein or DNA weight respectively, and about one half when expressed per unit of whole animal weight.

The results may be explained by the different protein and DNA content of the organs studied, although the values correspond to samples from filtered crude homogenates, which are not absolutely equal — but very similar — to those that can be found in the whole intact organ. In any case, the protein/DNA weight ratios for the three tissues studied are 28.6, 95.4 and 37.9 for liver, muscle and kidneys respectively, indicating a considerably larger cell size for muscle.

All the expressions used have obvious drawbacks. The expression of enzyme activity in relation to the tissue weight is not adequate for comparing different entities with different overall composition (i.e. adipose tissue, which contains a huge amount of lipidic reserves as compared with other tissues). Thus, this mode of expression could not be suitable for comparison with tissues not hampered by the weight of inactive or «diluting» structures or reserves. The expression of activity per unit of protein weight does not take into account the differences in content of structural or enzyme protein fractions; i.e. muscle contains a considerable amount of contractile proteins that in some way tend to «dilute» the other enzymes and make it rather difficult to compare readily the data obtained in this tissue with those found in the other tissues with other activities. The expression of enzyme activity per unit of DNA weight gives a more physiological view of the data, as it refers more or less directly to the cell unit. Nevertheless, there are considerable differences in cell size among the tissues and in different experimental situations when there can be induced changes both in cell size or in cell numbers, that affect the DNA content of the tissue, thus changing the expression of the enzyme activity. The expression of the enzymatic activity per whole organ referred to an uniform weight of the animal has also obvious drawbacks, as it does not take into account the relative blood irrigation and/or metabolic activity of the

organ studied. The main advantage of this mode of expression of enzyme activity over the other three studied here is, however, that it shows the relative importance of a reaction in a given organ at the level of the whole organism.

The general conclusions that can be drawn from the data shown in this paper are the necessity for multiple expression of the results of enzyme activities attained in any given experiment, as the general conclusions drawn from only one of them would not grant totally the possible conclusions extracted when comparing changes of activity in different tissues or organs.

Under the basis of these considerations, it is suggested that the results of measured enzymatic activity in any given experimental setup should be expressed in more than one form. The use of repeated expressions of the data could not, then, be considered as redundant, as the information drawn from the whole set is considerably more complete than the data shown in only one of the modes indicated, allowing a more physiological picture of the possible metabolic role of the enzyme activity studied.

Resumen

Se discuten las expresiones de actividad enzimática más utilizadas en estudios comparados de actividad en diferentes tejidos: microkatales por unidad de peso del tejido, por unidad de peso de proteína y por unidad de peso de ADN. Se utiliza también la expresión de microkatales presentes en un órgano determinado referidos a unidad de peso del animal, 100 g en el caso de la rata. Las diversas expresiones se han aplicado a los niveles de aspartato transaminasa, glutamato deshidrogenasa y AMP desaminasa en hígado, músculo estriado de pata trasera y riñones de rata adulta. De los datos presentados se concluye que las mediciones de actividades enzimáticas en tejidos deben ser expresadas en más de una forma, ya que la información obtenida a partir de una sola de ellas puede ser substancialmente distinta de la obtenida con otra de ellas, dando lugar a posibles conclusiones erróneas del papel metabólico jugado por el enzima en un tejido determinado.

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