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A Method for the Estimation of Striated Muscle Mass in Small Laboratory Animals *

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A method for the determination of the total muscle mass in small experimental animals is presented. Muscle proteins were quantified in the 1 M LiCl-soluble and distilled water-insoluble fraction of the eyeless, brainless, eviscerated and skinned carcass, as compared with a striated muscle sample from the same animal used as standard and processed in the same way as the whole carcass. The non-muscular tissues left in the carcass do not interfere with the final results. The total muscle mass in adult rats was estimated as 42.0 ± 2.8% of the *in vivo* weight.

There has been in the last years considerable work done on the general role of muscle in the metabolic economy of mammals. This interest is mainly centered on its role as substrate source for splanchnic gluconeogenesis, exemplarized by the CORI cycle (2), and the glucose-alanine cycle (4). There is, however, an important drawback in assessing its quantitative implication on the economy of the animal: the imprecise determination of total muscle mass. This problem limitates the quantitative studies of *in vivo* muscle function to very special situations as are the arterio-venous differences in amino acids and glucose in limb or chirurgically exposed muscle masses (5) with the incidence of artifacts due to the presence of other tissues as adipose, skin, etc.

The assessment of relative muscle mass in the whole body has been attempted by different ways, such as differential determination of ^{40}K or other mineral

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components (1, 3, 7), direct carcass weight, density estimation (8) and others (15).

Generally, the weight of eviscerated, skinned carcass has been used as an index of muscle weight; but these methods are subjected to the constant incidence of artifacts due to the interference of bone, bone marrow, cartilage, adipose and nervous tissues and blood.

It has been intended here to develop a method for the more accurate determination of the body's muscle mass in small mammals, based on the determination of the specifically high muscle content in myofibrill protein, mainly myosin. These proteins have been repeatedly isolated due to their solubility in concentrated saline solutions and insolubility in low ionic force media (6, 11, 12).

Materials and Methods

Healthy Wistar albino rats weighing 180 to 235 g were used. Animals were killed by decapitation, and after bleeding were dissected, and all viscerae, testicles, brain and eyes were removed. The rats were skinned and blotted, and the finger tips were clipped out. A muscle representative sample, devoid of macroscopic adipose tissue contamination, was obtained by cutting a piece of about 0.1-0.3 g of the muscle mass in the upper frontal part of the hind legs, above the knee. This sample contains a mixture of red and white muscle fibres, beig constituted by parts of *rectus femoris, vastus medialis* and *fasciae latae* muscles.

The gutted skinned carcass and the muscle sample were separately covered with aluminium foil and kept frozen at —30° until processing. The samples were weighed, and the carcass was chopped and passed twice through a common meat-grinder. The carcass paste was then thoroughly mixed, and an aliquot of about 0.2-0.4 g was taken with a spatula and was weighed. The muscle sample was chopped and minced with a knife blade and the sample was then weighed. Both samples were then separately homogenized in a glass-teflon motor-driven Potter-Elvejhem type homogenizer in 10 volumes of 1 M LiCl bufferd to pH 7.5 with 0.05 M phosphate buffer, in order to solubilize most of the structural protein in the tissue. After centrifugation, the insolubilized residue was again homogenized in 10 volumes of the extraction medium. The residue was washed again (together with the homogenizer) with the same solution. All supernatants and washings were combined and brought up to a final volume of 25 ml with 1 M LiCl in 0.05 M phosphate buffer.

A 1 ml aliquot of this protein solution was placed in a 22 × 220 mm tube. 20 ml of distilled water were added, dropping the LiCl concentration to 0.05 M, and causing the partial precipitation of the muscle structural protein. After standing overnight in the cold room (4° C), the tubes were decanted and the precipitate recovered by centrifugation. The precipitates were solubilized in 1 M NaOH by heating 10 minutes at 100° C, and aliquots were used for protein determination with the Folin-phenol reagent method (10, 13), using defatted bovine serum albumin as standard.

The protein content in the muscle extracts were correlated to the original chopped muscle weights, according to the following calculations:

R = *in vivo* rat weight.
C = «Clean» carcass weight (minus the weight of the sample S).
S = Muscle sample weight used for homogenization.
P = Carcass paste sample used for homogenization.
z = mg of protein in muscle sample water precipitate.
y = mg of protein in carcass paste sample water precipitate.

Ratio of «precipitable proteins» in the muscle sample: z/S.
Ratio of «precipitable proteins» in the carcass sample: y/P.
Fraction of «muscle» content in the whole carcass: yS/zP.
Total «muscle» content in the rat: ySC/zP + S.
«Muscle» content (%) in the rat: (100/R) × (ySC/zP + S).

Results and Discussion

The use of a muscle sample from the same animal as internal standard for the determination of muscle mass, allows us to correct the differences in weight and structural protein content in the fraction studied.

In rats weighing 205.1 ± 6.9 g, the muscle mass averaged 42.0 ± 2.8 % of the rat *in vivo* weight (n = 7), while the whole carcass weight was of 51.1 ± 0.8 % of the rat *in vivo* weight. Thus, there is an approximate «constant» error of about 20 % in muscle mass if the skinned eviscerated carcass figure is used instead of the calculated muscle content.

The weight ratios of precipitable proteins in samples of *soleus* and *extensor digitorum longus* muscles were not statistically different between them and contained the same average fraction of low ionic strength precipitable protein as the hind leg muscle sample used in our method. This is in agreement with the known constancy of the myosin fraction in different muscles, 34-38 % of striated muscle protein (9).

In order to check possible sources of error, the same procedure outlined here was followed using other different tissues of the animals, being the equivalence in «muscle» content expressed as percentage of the weight of the sample in table I.

The potentially most interfering tissues are: skin, adipose tissue, cartilage, nervous tissue, bone, bone marrow, and blood. However, none of these constituted an important source of error, as their «muscle» content is very low and their presence in the carcass is not too much important. It is assumed then, that the presence of minute amounts of these tissues — or even a considerable proportion as is the case with bone —, will have a low incidence in the total muscle figure, probably lower than the weighing and mixing errors inevitably introduced by the long procedure.

Table I. *Structural protein content (distilled water-insoluble fraction) in different tissues of normal adult rats.*
«Striated Muscle» equivalence of the different tissues are given as percentage of their weight.

Organ or tissue	mg protein/g	«Striated muscle» content (%)
Muscle sample	27.2	100.0
Carcass paste *	20.4	75.0
Brain	6.1	22.4
Ear cartilage	2.4	8.8
Adipose tissue	2.3	8.5
Skin	2.2	8.1
Bone+bone marrow	0.7	2.6
Blood	0.3 **	1.1

* Contains muscle, bone, bone marrow, adipose tissue, cartilage and fragments of skin and nervous tissue.
** mg of protein/ml of blood.

The percentages of muscle mass versus body mass obtained for adult rats agree in a general way with other data published (16) and with data obtained from humans (14), comparable with those of the rat because of the constancy of muscle protein proportion versus body weight in terrestrial vertebrates (16).

Resumen

Se presenta un método para la determinación de la masa muscular total de pequeños animales de laboratorio. Se determinó el contenido en proteína del precipitado obtenido por la adición de agua destilada a un extracto (en ClLi 1 M) del homogenado del canal eviscerado y despelle-

jado, al que se habían sacado además los ojos y el encéfalo; comparándose la cantidad de proteína obtenida en este precipitado con la obtenida en un fragmento de músculo estriado del mismo animal tratado de la misma manera y utilizado como patrón. Lo tejidos no musculares que quedan en el canal no interfieren en los resultados finales por su bajo contenido en proteínas en esta fracción. La masa muscular total hallada en ratas adultas resultó ser del orden del 42,0 ± 2,8 % del peso del animal *in vivo*.

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