THE PROTEIN CONTENT OF MUSCLE.

By N. W. JANNEY.

(From the Chemical Laboratory of the Montefiore Home and Hospital for Chronic Invalids, New York.)

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An exact knowledge of the composition of animal muscle is of considerable importance for the science of nutrition. Though the proteins represent the most important muscle constituents, the usual means employed to determine the amount of these substances present remain rather inaccurate. Thus the amount of protein in muscle is still most commonly estimated by multiplying the total nitrogen by the classical protein factor 6.25. This mode of calculation is used in most of the standard food analyses tables, such as published by König,[1] Atwater and Bryant,[2] Atwater,[3] and Lusk.[4] On the protein values so obtained are based various metabolic data including calculations of the caloric values. Although this method of estimating muscle protein is generally known to be a faulty one, its inadequacies may still be alluded to in the hope of lessening its present extensive employment. The protein factor 6.25 is based on acceptance of 16.00 per cent as the nitrogen content of muscle proteins. Although many of the older nitrogen analyses of muscle proteins do give values averaging about 16.00 per cent, the muscle protein preparations then analyzed were of very dubious purity. More recently Osborne and his coworkers have reported analyses of carefully prepared animal muscle proteins which are considerably higher than 16.00 per cent. Similar analytical results have also been obtained in the writer's laboratory for pure muscle proteins. The nitrogen content was found to be

[1] König, J., Chemie der Menschlichen Nahrungs- und Genussmittel, Berlin, 4th edition, 1910, iii, 252.

[2] Atwater, W. O., and Bryant, A. P., *U. S. Dept. Agric., Bull. 28,* 1906.

[3] Atwater, W. O., *U. S. Dept. Agric., Farmer's Bull. 142,* 1906.

[4] Lusk, G., The Elements of the Science of Nutrition, Philadelphia, 2nd edition, 1909.

rather constant for various species of higher animals, ranging from 16.2 to 16.7 per cent in many analyses. In view of this work the protein factor 6.25 can no longer be considered accurate. Moreover, a very considerable error is introduced by using the total nitrogen of muscle in this mode of calculation of the protein, for our analyses have shown that about 13 per cent of the total nitrogen is combined in non-protein substances in muscle. The amount of protein obtained is greatly increased in consequence.

A further source of confusion and error is the loose application of the term "protein" to include practically all protein and "nitrogenous non-protein substances" found in animal muscle. Thus Atwater and Bryant, who adopt this nomenclature, calculate the number of calories obtainable from the proteins of meats from protein in this sense. As extractive substances of non-protein nature are here reckoned in, the result must be inaccuracy in the number of calories ascribed to the meat protein.

Another much used indirect method for estimating the amount of muscle protein is that "by difference." According to this plan the total water-free substance is ascertained; and from this value the solid material found in the ether extract of the dried muscle, together with the ash, is subtracted. The result is designated as protein and varies but slightly from that obtained by multiplying the total muscle nitrogen by the factor 6.25. This correspondence is, however, no criterion of accuracy, for nearly the same sources of error are present in both procedures. The "protein" as thus ascertained from anhydrous muscle material includes also the non-protein extractives aside from those soluble in ether. In the article immediately preceding mention has also been made of the fact that it is very difficult to free dried muscle entirely of fatty substances by means of ether extraction. Any residual amount of fat thus remaining in the extracted protein would serve to increase the protein content obtained by this mode of calculation. This presents an additional source of inaccuracy. Lastly the carbohydrate content of muscle is likewise reckoned as protein by this procedure. A further small but appreciable error is thus introduced.

To enter into a discussion of various other methods proposed for the estimation of muscle proteins is scarcely advisable in view of the fact that none have come into very general use. The chief

reason for this is that these procedures are of questionable accuracy in nearly all cases.

These considerations have led the writer to devise a new method for the determination of the muscle proteins which permits of a rather exact determination of these substances. The improved technique of this method is described in the preceding article.

In the table are arranged for comparison protein values taken from Atwater and Bryant's food analyses obtained by multiplication of the total muscle nitrogen by the factor 6.25, as well as "by difference," as described above. Average results of a number of the writer's analyses appear also. All the analyses refer to the same kind of muscle as indicated. In the majority of cases

Comparison of Calculated and Actual Amounts of Protein in Muscle.

Species.	Total nitrogen in muscle.	Protein.		
		N × 6.25.	By difference.	Average of analyses according to the writer.
	per cent	*per cent*	*per cent*	*per cent*
Chicken	3.09*	19.3	19.0	16.6
Fish (halibut)	2.98*	18.6	18.4	16.5
Ox	3.46	21.6	21.5	16.6
Rabbit	3.39	20.8		16.3
Cat	3.38	21.1		17.8
Dog	3.25	20.2		17.4
Man	3.15	19.7		16.4

*Calculated from Atwater and Bryant's protein values. The remaining nitrogen analyses are by the writer.

the nitrogen was estimated in the same sample of muscle for which the protein was determined according to the writer's method. *The amount of protein calculated is seen to exceed that determined by actual analysis by about 15 to 20 per cent in nearly all cases. Continuance of the use of these usually employed methods of calculating muscle protein should therefore be discouraged.*

In order to put calculations of the caloric values of meat proteins on a more exact basis, the calories yielded by the pure muscle protein obtained in the analytical method should be determined. As it is possible to make preparations of meat extractives without

appreciable loss,[5] the caloric value of such preparations can also be obtained and correspondingly allowed for in metabolic work of this description.

The table also brings out the interesting fact that the actual protein content of muscle is usually quite constant for various species, even those as far separated zoologically as man and fish. In wasting diseases among human beings, however, values as low as twelve have been recorded. It seems possible that in such muscular disorders as myasthenia gravis the amount of protein muscle substance present might be found to bear a definite relation to the specific muscular seat of the disease.

[5] Janney, N. W., and Csonka, F. A., *J. Biol. Chem.*, 1915, xxii, 195.