CLXVI. A NOTE ON QUANTITATIVE METHODS OF MEASURING THE NUTRITIVE VALUE OF PROTEINS.

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IN an article appearing recently in this *Journal*, Kon [1928] has applied to the study of the nutritive value of the protein tuberin, the globulin of the potato tuber, the method elaborated by the author [1924], involving a study of the nitrogen metabolism of young growing rats under controlled conditions of feeding. In comparison with this method, analogous results obtained by a quantitative growth method devised by Osborne, Mendel and Ferry [1919] were also presented. According to the first method, the nutritive value of the protein is taken as the total nitrogen retention of the experimental animal in percentage of the total absorbed nitrogen. This percentage is called the "biological value" of the protein, a term introduced by Thomas [1909]. According to the second method, the nutritive value of the protein is measured by the gain in body-weight of the experimental animal per gram of protein consumed.

In discussing the significance of the results secured by these two methods, Kon makes certain statements to which exception may be taken. Speaking of the nitrogen balance method, he says (pp. 266-7): "A specific error seems to be inherent in the method . . . namely, the tendency to give higher biological values in the periods immediately following the standardising nitrogen-free or low-nitrogen periods. This is especially marked in the present work, but may also be found in the papers of Mitchell and his co-workers, especially Mitchell and Beadles [1927], and of Klein, Harrow, Pine and Funk [1926]."

If this were true it would constitute a serious criticism of the method. Among the first applications of the method in the author's laboratory, the possibility of such an error was anticipated and specifically studied by Mitchell and Carman [1924], with results quite contrary to the conclusion of Kon. It should be realised in a consideration of this question, first, that discrepancies will generally exist between duplicate determinations of the biological value of a specific source of protein obtained with two groups of rats or even with the same group of rats, and, second, that if one of the duplicate determinations is made in the period following a period of low-nitrogen feeding, the chances are even that it will be larger than the other determinations not so made,

even though the period of nitrogen underfeeding has exerted no effect on the utilisation of the dietary protein. In other words, the question is a statistical one, and cannot be settled by citing isolated instances in which experimental data *appear* to indicate an increased biological value following nitrogen underfeeding. The results of Kon obtained with tuberin are of this description, but neither they, nor the other results cited, are sufficient to prove the point at issue, since they have been selected from a number of other results of an opposite significance. Thus, in the work of Klein *et al.* [1926], wheat bran gave an average biological value of 60 following a period of nitrogen-free feeding, and one of 54 in another type of period, but corn meal gave very poor and variable results (averaging only 18) in a period following a standardising period, and an average of 56 in another period not so located. The same situation exists in the work of Mitchell and Beadles [1927] to which reference is also made.

It seems clear that the best solution of the question can be obtained only from a survey of all published data that might bear upon it. Besides the Illinois data, there are only those data from Cambridge and Warsaw already cited. The following table summarises all of the data obtained from the author's laboratory at the University of Illinois.

The effect of a period of low-nitrogen feeding on the biological value of protein obtained in an immediately following period.

Source of protein	Average biological values obtained		Excess of (1) over (2)	Reference
	Following low-N feeding (1)	Not following low-N feeding (2)		
Whole egg	92	94	-2	Mitchell and Carman [1924]
Whole wheat	63	68	-5	" " "
Cocoa + milk	73	65	+8	Mitchell, Beadles and Keith [1926]
Whole egg	90	99	-9	Mitchell and Carman [1926]
White flour	56	48	+8	" " "
Egg albumin	86	81	+5	" " "
Egg albumin	82	81	+1	" " "
White flour	48	54	-6	" " "
Veal	64	59	+5	" " "
White flour	49	49	± 0	" " "
Beef + white flour	70	76	-6	" " "
White flour	55	55	± 0	" " "
Beef liver	78	75	+3	Mitchell and Beadles [1927]
Beef heart	84	68	+16	" " "
Beef heart	76	69	+7	" " "
Beef liver	73	75	-2	" " "
Pork tenderloin	79	74	+5	Mitchell, Beadles and Kruger [1927]

Among 17 possible comparisons, 9 favoured the view that low-nitrogen feeding increases the biological value in an immediately subsequent period, 6 contradicted this view, and 2 gave a non-committal answer. The average difference in biological value for the 17 cases was +1.6, in favour of the value obtained after low-nitrogen feeding. As the method is applied in the author's laboratory, there seems to be no reason to believe that a period of low-nitrogen

feeding exerts any but an inappreciable effect upon the utilisation of protein in a subsequent period.

Kon is also in error in his attempt to explain differences in the nutritive ratings of proteins as obtained by the author's method and by the method of Osborne, Mendel and Ferry. On this point he says: "Obviously the two methods are not strictly comparable, as the one measures the efficiency of a given protein to make good, over short periods of time, the wear and tear of body tissues, while the other measures the ability of a protein to build new tissues."

This constitutes a misinterpretation of the significance of results secured by the author's method, a misinterpretation that can be readily refuted by any of the calculations made by Kon himself. Take the data of Rat 1 on the tuberin ration, for instance. This rat consumed daily 104 mg. of tuberin-nitrogen and excreted daily in the faeces 23 mg. of nitrogen, of which 22 mg. were estimated to be of body origin (metabolic nitrogen). Hence, only 1 mg. of dietary nitrogen was actually indigestible, and the absorbed nitrogen, therefore, amounted to 103 mg. daily. The daily urinary nitrogen amounted to 45 mg., of which 20 mg. was estimated to be of body origin (endogenous) and only 25 mg. represented unutilised dietary nitrogen. Hence, of the 103 mg. of tuberin nitrogen absorbed 25 mg. was wasted and 78 mg., or 76 % (the biological value), was retained in the body. According to Kon, this 78 mg. was used only to make good the wear and tear of body tissues. But this rat was growing and storing daily 36 mg. of nitrogen ($104 - 23 - 45 = 36$), which must be an integral fraction of the total retention of 78 mg., since no other source of nitrogen was available. The latter value includes all functions, maintenance, growth, and the replenishment of the digestive glands—for which the dietary nitrogen is being used.

Thus, the biological value of a protein measures that fraction of the absorbed protein nitrogen that is being used in all of the anabolic reactions of the body. On the other hand, the gain per gram of protein consumed obtained under the conditions prescribed by Osborne, Mendel and Ferry credits to the dietary protein only that fraction of the protein intake that is being used for growth. Hence, this measure of the nutritive value of a protein is affected, not only by the amino-acid make-up of the protein itself and the unknown physiological factors that determine its utilisation in anabolism, but also by the ratio of protein consumed to the size of rat used. The larger this ratio becomes, the smaller will be the fraction of the nitrogen intake needed to make good the endogenous losses of nitrogen, and the greater the fraction that is available for growth, and, hence, for the same source of protein, the greater the gain per gram of protein consumed. For this reason, this measure of protein value is subject to variation caused by variable food intakes. If rats of equal size are employed and the food intakes are comparable, the measures of protein efficiency obtained for different proteins will be comparable. But it is a hazardous undertaking to compare gains per gram of protein consumed

obtained in different experiments without reference to the size of animals employed, or to the amounts of food consumed, as Kon and others before him have done.

Hoagland and Snider [1926], in one of their first applications of the method of Osborne, Mendel and Ferry to the study of the nutritive value of proteins, noted that, throughout a number of ration comparisons, the gain in weight per gram of protein consumed was in the large majority of cases greater for the male than for the female rats. This sex difference was correctly interpreted as the result of the greater growth capacity of male rats and the resulting greater consumption of food in proportion to weight. Hence, with the males a larger proportion of the protein consumed was available for growth and was so used. Although in their later work Hoagland and Snider used only male rats, the complete significance of these early experiments has not been appreciated, *i.e.* that in comparisons of different sources of protein a great disparity in the food consumption of experimental rats, whether because of differences in sex or of the growth-promoting value of the rations, will always obscure the interpretation of the observed differences in the ratio of gain to protein consumed.

There is another reason why the results obtained by the author's method for a series of proteins will differ from those obtained by the numerical method of Osborne, Mendel and Ferry. In the former method differences in the digestibility of proteins are not involved in the biological values obtained, while in the results of the latter method they are. For two proteins of equal biological value, the gain in weight per gram consumed will be the greater for the more digestible protein. This is one reason why animal proteins are graded so much higher than cereal proteins by this method than they are by their respective biological values.

Either method is capable of yielding accurate comparisons of the nutritive values of different sources of protein, but only when the necessary experimental conditions are imposed, as dictated by a critical study of all factors that may affect the final results. However, the methods are not equivalent and the results obtained need not parallel each other.

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