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This Week's Citation Classic®

Bender A E & Miller D S. A new brief method of estimating net protein value. *Biochemical J.* 53:vii, 1953; and **Miller D S & Bender A E.** The determination of the net utilization of proteins by a shortened method. *Brit. J. Nutr.* 9:382-8, 1955.
[Crookes Laboratories Ltd., London, England]

The classical Thomas-Mitchell bioassay of protein quality using growing rats consists of subtracting the excreted urinary N from the dietary intake of protein N and so calculating the proportion assimilated. Three proteins could be evaluated in a five-week period. The abbreviated method consists of analysing the carcass to determine the amount of N assimilated and allows the assay of seven proteins in a 10-day period. [The *SCI*® indicates that these papers have been cited in over 100 and 395 publications, respectively.]

"Sheer Ignorance?"

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It is pertinent to recall that people were assessing protein quality[1,2] before all the essential amino acids had been discovered (threonine 1935)—hence the air of mysticism around the coterie of specialists who dabbled in protein quality.

There has long been a fundamental problem in this area, namely, How much of the excreted N comes from degradation of tissues and is therefore not related to the diet? Thomas measured this by analysing urine N produced on a diet free from protein, but it is questioned whether this is the same when protein is fed. H.H. Mitchell attempted to solve the problem by feeding, instead of a protein-free diet, a low level of a completely assimilated protein (4 percent dried egg), which would not appear in the urine. But is this the same figure as when test proteins are fed at the usual 10 percent dietary level?

We carried out a large number of assays by the Thomas-Mitchell method, and when we discussed this problem with our director of research, Dr. J.I.M. Jones, he offhandedly said, "Get rid of that term from the equation." My late colleague, Derek Miller, did so over the weekend by deriving from the original Thomas-Mitchell equation one that measured what is in the carcass as distinct from the difference between intake and output. In fact, we had only removed it from the equation, not from the assay.

My part was to develop the methodology—in three parts. As an inspired (or lucky) guess we took four litters each of eight rats and grouped them into eight weight-matched groups of four. One group was fed a protein-free diet, leaving seven test groups.

The second guess was to feed for 10 days. One other abbreviation was introduced. C.R. Moulton[3] had shown that at a given age body constituents, including N and water, were constant, so instead of measuring carcass N each time, we could derive this from the body water.

The third guess was to determine water by drying the carcasses at 105° C for 48 hours (actually over the weekend, when we could avoid the smell). Some years later Rafalski in Poland tested out every possible permutation of numbers of animals from 1 to 17, length of trial period from 4 to 20 days, and conditions of drying and concluded that our original conditions were the "best."

Determining the N:water ratio at various ages called for hundreds of determinations of N (Kjeldahl method) and water in rats aged from 0 to 503 days of age. In time the condensed sulphuric acid vapours literally brought down the ducts from the fume cupboards.

One intriguing finding came to light. The N:water ratio increases from about 2.0 at birth to 4.8 at "chemical maturity"—70 days of age. When this curve was extrapolated backwards, it met the horizontal axis at minus 22 days of age—the date of conception of the fetus. We confirmed this plot with a single determination of the N:water ratio of a fetus of known age. So the rat would appear to start life as a drop of water and then steadily increase the N!?

One overall result was that a Spanish nutritionist, Professor Gregorio Varela of Madrid, was introduced to our method[4] while on sabbatical leave in Cambridge, and the outcome was an honorary DSc from the University Complutense of Madrid in 1983.

N.B. Sheer ignorance led to an error in the title of the original paper. "Net protein value" had already been defined as NPU x protein content—it should have been net protein utilization.

1. **Mitchell H H.** A method for determining the biological value of protein. *J. Biol. Chem.* 58:873-903, 1924. (Cited 100 times since 1945.)
2. **Osborne T B, Mendel L B & Ferry E L.** A method for expressing numerically the growth promoting value of a protein. *J. Biol. Chem.* 37:223-9, 1919. (Cited 250 times since 1945.)
3. **Moulton C R.** Age and chemical development in mammals. *J. Biol. Chem.* 57:79-97, 1923. (Cited 215 times since 1945.)
4. **Moreiras-Varela O, Ruiz-Roso B & Varela G.** Influence of CFR (cooking, freezing, reheating) system on the nutritive quality of food protein. (Zeuthen P, Cheftel J C, Eriksson C, Jul M, Leniger H, Linko P, Varela G & Vos G, eds.) *Thermal processing and quality of foods.* Amsterdam, The Netherlands: Elsevier Applied Science, 1984. p. 899-902.