

**CXCIX. THE BIOLOGICAL VALUES OF PROTEINS.  
I. A METHOD FOR MEASURING THE NITROGENOUS  
EXCHANGE OF RATS FOR THE PURPOSE OF DETER-  
MINING THE BIOLOGICAL VALUE OF PROTEINS.**

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THE metabolism cages used were those described by Ackroyd and Hopkins [1916] with the detachable wire floor introduced by Boas [1924] and glass separators of Gross and Connell [1923] to effect quantitative separation of urine and faeces.

The method consisted in a determination of the average daily nitrogenous balance sheet on a diet containing a definite proportion of the protein under investigation. Two adult male rats (250 g. weight upwards) were placed in each cage, and determinations made of the total nitrogen ingested and of that excreted in urine and faeces respectively, during a period lasting 4 or 5 days.

The cages of other experimental animals and reagent bottles containing ammonia were removed from the room in which the metabolism cages were kept to avoid any error due to absorption of small quantities of ammonia.

*The control of the intake of nitrogen.*

The dry constituents of the experimental diet were carefully mixed together and made into a stiff paste with distilled water, samples being removed for determinations of nitrogen. An amount of the diet, more than sufficient for the whole experiment, was put in a wide-necked stoppered bottle, kept in the refrigerator room when not in use. From this stock the approximate amount needed every day was weighed into the daily ration pots, mixed to a creamy consistency with water and placed in the chambers at either end of the two arms of the metabolism cage. The administration of the diet in the form of a liquid paste was a device introduced by Korenchevsky (unpublished experiments) in this laboratory to prevent the scattering of food. Care was taken to gauge the appetite of the rat with sufficient accuracy to avoid a large residue, any traces of food left being collected at the end of the experiment and the nitrogen in it estimated. The diet bottle was weighed before and after the experiment to obtain the total amount of diet removed. The corresponding amount of nitrogen was calculated and this, after correction for the nitrogen contained in the uneaten residue, gave the total intake.

*The control of the nitrogen output.*

The urine and faeces were collected daily. To prevent decomposition of urine and consequent loss of ammonia 5 cc. of a 5 % solution of carbolic acid and 1 cc. of a 10 % solution of thymol were placed in the beaker used to collect the urine. During the daily collection of excreta and washing of the metabolism cage the rats were placed in small cages of perforated zinc supported by means of zinc arms over large porcelain dishes, from which any urine and faeces passed during this period could easily be collected.

The faeces were transferred daily from the collecting beaker into a weighed porcelain crucible provided with a lid. If soiled with urine, they were previously washed and the washings added to the urine bottle. The faeces crucible, like the urine bottle, was kept in the refrigerator room when not in use. At the end of the experiment the faeces were moistened with a few drops of a 10 % solution of oxalic acid, dried at 110° for 12 hours, cooled in a desiccator, weighed and then powdered with a pestle, the hair often contained being chopped up with scissors to obtain a uniform mixture. The powdered faeces which are extremely hygroscopic were then transferred to a weighing bottle and dried again at 110°. After cooling in a desiccator aliquot portions were weighed out for nitrogen determination.

The metabolism cage, funnel, separator and beakers placed for collection of faeces and urine respectively were washed down daily with dilute acetic acid and distilled water, using a large brush and a glass rod tipped with rubber. The use of acetic acid prevented risk of ammonia loss during the process of washing and helped to remove traces of solid deposit; a little alcohol was also found useful in removing traces of fat. Urine and washings were filtered through muslin into a large weighed stoppered bottle. The hair removed by this filtration was analysed separately for nitrogen, until a number of analyses had shown that the amount concerned was too small to be significant. At the close of the experiment the urine bottle was weighed and aliquot portions removed for nitrogen determinations.

The nitrogen estimations were made by Kjeldahl's method.

*Risk of nitrogen loss during the experiments.*

It seemed possible that a loss of ammonia from urine might occur owing to the relatively small volume passed (*ca.* 20–30 cc. daily from 2 rats of 300–400 g. weight each) and the large surface of the cage and funnel, both being about 10 in. in diameter. The result of the following test, devised to simulate an actual experiment, showed, however, that no such risk existed. Urine, of known nitrogen content, was allowed to drop from a burette at intervals during the day and trickle over an empty cage, funnel and separator, being finally collected below in a beaker. After 24 hours the whole apparatus was washed as described above and nitrogen estimated in the collected urine and washings. The amount of urine dropped was 50.2 cc., calculated to contain

0.256 g. nitrogen; the nitrogen recovered from the collected urine and washings was 0.256 g.

In a second test 81.2 cc. urine, containing 0.442 g. nitrogen, was allowed to drop at intervals over 3 days, collection of urine and washings taking place daily. The total nitrogen collected was 0.440 g.

*Estimation of the biological value of proteins.*

The above method can be used to determine the daily nitrogen balance sheet of any pair of rats maintained on a diet containing a known proportion of any given protein (P).

To estimate the biological value of this protein, it is necessary to determine the minimum amount of nitrogen (M) (derived from the given protein as sole source of nitrogen in the diet) which must be absorbed to compensate the daily nitrogenous expenditure (E) on a nitrogen-free diet. The latter is determined for the same pair of rats, being the total nitrogen excreted in faeces and urine on a protein-free diet consisting of fat and carbohydrate only. The biological value of protein P can then be defined as  $100 \times \frac{E}{M}$ .

The principal technical difficulty lies in the determination of E. Appetite soon fails on diets devoid of nitrogen, sometimes in the first few days of the experiment. It is, therefore, often difficult to secure a sufficient calorie intake to prevent loss of weight. If there should be any significant loss of weight, E will be over-estimated.

The provision of B vitamins is also necessary to maintain appetite and their administration involves the addition of small amounts of extra nitrogen to the diet. By the use of purified concentrates from yeast, however, these additions can be made very small.

A discussion of these complications, together with examples of the calculations involved, are given by Fixsen [1930], who used this method for determining the biological value of purified caseinogen.

REFERENCES.

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