CLXXIV. NOTE ON THE NITROGEN CONTENT OF CERTAIN PROTEINS.

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In the course of their investigations, many workers require to estimate small amounts of protein material; several methods are available for the determination of protein, but for small amounts perhaps the best is the determination of total nitrogen content, as on the micro-scale this is rapid, accurate and economical of material. But in order that this method shall give a true estimation of the amount of protein present, it is essential that the nitrogen content of the protein in question be accurately known.

In the standard textbooks, proteins are variously quoted as containing 15–16 % nitrogen, the generally accepted factor for the conversion of nitrogen to protein being 6·25. Hausmann [1899] found the nitrogen content of crystalline egg-albumin to be 15·51 %, that of crystalline serum-albumin to be 14·60 % and of horse serum-globulin to be 16·17 %. Osborne [1903] quotes the nitrogen content of caseinogen as 15·62 % and of egg-albumin as 15·51 %. He investigated the nitrogen contents of various vegetable proteins which he found to be higher than those of proteins of animal origin. His values [1924] for different vegetable proteins varied from 18·84 to 15·62 % with an average of about 17·3 %. Miller and Chibnall [1932] found a nitrogen content for various grass-proteins varying from 12·3 to 14·0 % of dry weight but their preparations admittedly contained non-nitrogenous impurities. Banzhaf [1914] estimated the nitrogen contents of various immune sera, some of which were in the crude state and some purified by ammonium sulphate precipitation. The values found for nitrogen were much lower than the earlier figures quoted above, varying from 14·07 % for purified antidiphtheria pseudoglobulin to 13·09 % for crude antitetanus serum. Felton [1932] on the other hand has found higher values for Felton protein, varying from 14·9 to 15·7 %.

In view of the large variation in results quoted above and also of the fact that 6·25 is still accepted by many workers as a universal protein-nitrogen factor regardless of the nature of the protein, we have thought it desirable to carry out nitrogen determinations on known weights of certain proteins encountered during investigations on toxins and sera.

Purification of the protein.

The method of purifying the protein varied according to the nature of the material.

Diphtheria toxin and toxoid. The culture filtrate was acidified with hydrochloric acid to p_H 4·6–4·7 (for toxin) or to p_H 4·2–4·3 (for toxoid). The mixture was left until a floccular precipitate began to form, and this was separated by means of a centrifuge, washed first with buffer at p_H 4·0 and finally with distilled water. It was then emulsified in water and taken into solution by the addition of the minimum quantity of sodium hydroxide, filtered to remove any extraneous solid matter and re-acidified. The precipitate was separated and washed as before and finally dried to constant weight over phosphorus pentoxide. The material thus purified contained 35–106 flocculation units per mg. of dry weight. For nitrogen determinations a known weight was dissolved in 0·7 % saline in quantity sufficient to bring the nitrogen content to 0·3–0·8 mg. per cc.

Diphtheria antitoxin and normal pseudoglobulin. The serum was fractionated with ammonium sulphate and dialysed. The pseudoglobulin was electrodialysed to remove any remaining euglobulin, which was filtered off. The clear filtrate was dried over phosphorus pentoxide to constant weight.

Serum-albumin. The globulins from the serum were removed by precipitation with ammonium sulphate and the filtrate was dialysed till free from ammonium sulphate and dried to constant weight over phosphorus pentoxide.

Purified type I pneumococcus antibody. The Felton protein was obtained, after a preliminary salting out of the globulins from the serum with ammonium sulphate, by dialysis of this fraction against tap and distilled water, and an isoelectric fractionation of the resulting mixture to remove euglobulin and pseudoglobulin. The euglobulin was precipitated at p_H 5·0 and separated from the liquid by centrifuging; the Felton protein was precipitated from the supernatant liquid containing pseudoglobulin and Felton antibody, at p_H 6·8. It was collected by means of a centrifuge and dissolved in 3 % saline. When required for the nitrogen determination the protein was dialysed from salt against distilled water, separated by means of a centrifuge and washed several times with distilled water. It was dried over phosphorus pentoxide to constant weight.

Method of nitrogen determinations.

Nitrogen determinations were carried out by a modification of Pregl's method [1924], a small scale method based on the classical one of Kjeldahl. Four to seven estimations were carried out on each protein sample, the whole process taking about an hour and a half. By this means we have been able to determine quantities of nitrogen of the order of 0·1 mg. with a standard deviation of $\pm$ 0·002 mg. In certain cases the standard deviation for a series of four determinations has been as low as $\pm$ 0·0005 mg. In fact, in the results quoted in Table I the error in weighing sometimes approximates to the error

in nitrogen determinations and the standard deviations quoted were computed by taking both errors into account. It is known that the usual Kjeldahl method of nitrogen estimation does not recover all the nitrogen from certain materials, and that to obtain full recovery the Arnold-Gunning modification must be used. In order to determine whether this was necessary for the materials in question, a number of estimations were made by our usual modification in parallel with estimations using the Arnold-Gunning modification. There was no difference in the amount of nitrogen found with the two methods.

Table I. *Nitrogen content of certain proteins.*

No.	Type of protein	Nitrogen %	Nitrogen factor
TMR 597	Diphtheria toxoid*	14·07 ±0·06	7·12 ±0·03
TMJ 822	,,	14·64 ±0·04	6·82 ±0·02
TV 658	Diphtheria toxin	14·27 ±0·13	7·01 ±0·06
TX 659	,,	14·43 ±0·10	6·92 ±0·05
SS 42	,,	13·62 ±0·23	7·32 ±0·11
TP 592	,,	14·05 ±0·22	7·11 ±0·11
TR 642	,,	14·26 ±0·07	7·01 ±0·03
TJ 652	,,	14·60 ±0·08	6·78 ±0·04
PC 1667	Purified antidiphtheria pseudoglobulin	13·74 ±0·21	7·35 ±0·10
PC 1744	,, ,,	13·57 ±0·04	7·37 ±0·02
PC 1760	,, ,,	14·53 ±0·31	6·88 ±0·15
PC 1749	,, ,,	14·93 ±0·08	6·69 ±0·04
RX 2008	,, ,,	14·16 ±0·18	7·05 ±0·09
	Normal pseudoglobulin	14·12 ±0·17	7·08 ±0·08
	Normal serum-albumin	13·82 ±0·50	7·23 ±0·25
NC 3	Purified type I pneumococcus antibody	14·10 ±0·07	7·09 ±0·04
	Mean of whole series	14·18	7·05

* The diphtheria toxins were prepared from cultures on the different types of culture medium. No significant difference in nitrogen content was found when different types of medium were used.

Discussion.

Of these 16 estimations on different proteins only one (PC 1744) gives a nitrogen factor definitely greater than 7·25 and only one (PC 1749) a factor definitely less than 6·80. The mean factor for the whole series is 7·05, corresponding to 14·18 % nitrogen. The divergence between the upper and lower factors given is only slightly greater than our usual error of nitrogen determination. This is a markedly lower nitrogen content than the older results quoted by Osborne or Hausmann but is in excess of most of the results quoted by Banzhaf for immune sera or by Miller and Chibnall for grass-proteins. A nitrogen content might appear to be greater than the true value if the material were contaminated with impurities richer in nitrogen than the true protein, such as certain amino-acids, amides, ammonium sulphate, *etc.* High nitrogen values would therefore result if precautions were not taken to separate the proteins from such impurities. The values obtained would on the other hand be too low if the protein were contaminated with non-nitrogenous impurities such as carbohydrates, salts, dust or silica. They would also be low if the nitrogen were not recovered quantitatively by the Kjeldahl method.

In order therefore to assess the reliability of the results obtained it is necessary to examine very carefully the methods used in the preparation and analysis of the protein material.

Many proteins are prepared by fractionation of the original material with ammonium sulphate. If this salt is not removed completely by subsequent dialysis, error will be introduced. In the preparation of the pseudoglobulin, albumin and Felton protein used by us, ammonium sulphate was used but great care was taken to ensure its complete removal by dialysis. The fact that no significant difference in nitrogen content was obtained for serum-albumin, Felton protein, antidiphtheria and normal pseudoglobulin and diphtheria toxin, proteins derived from very different sources and purified by different methods, makes it improbable that there is any appreciable error in the results due to contamination of the purified product with impurities, nitrogenous or otherwise. As regards the recovery of nitrogen by the modified Kjeldahl method employed we have tested the recovery of nitrogen from solutions of ammonium sulphate, urea and asparagine. The results are usually about 0·4–0·5 % below the theoretical so that it is probable that the percentages of nitrogen to dry weights quoted in Table I ought to be raised by 0·4–0·5 %.

It thus appears improbable that the values quoted in the table differ from the true values for the types of protein investigated by amounts greater than the deviations given.

Summary.

The figures for nitrogen content of various proteins usually quoted vary from 15 to 16 %, or even higher. The nitrogen content of certain proteins derived from diphtheria toxin, and from diphtheria and other sera, when determined by the micro-Kjeldahl method is considerably lower, varying from 13·57 to 14·64 %. The average is 14·18, giving a nitrogen factor of about 7·05.

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