2. The vitamin B_1 content of Majestic tubers at the time of lifting (September) is about 140 μg./100 g.

3. During the growing season the vitamin B_1 content of tubers increases continuously up to the time of lifting of maincrop potatoes.

4. The vitamin B_1 content of stored tubers falls during the winter and spring; the greater part of the loss takes place in spring, and is apparently due rather to sprouting than to storage, especially as white sprouts have a higher vitamin B_1 content than the tuber which produces them.

5. The vitamin B_1 content of young potato leaves is very high.

6. An adjuvant factor of undetermined nature is present in the centres of tubers between April and August, and always present in the skin layer.

7. Green sprouts completely inhibit the growth of the fungus.

I should like to express my thanks to the following: to F. C. Bawden, under whose auspices the work was carried out, and N. W. Pirie, for advice; to P. H. Gregory for potato samples and help in planning the experiments; to H. M. Sinclair for a culture of *Phycomyces blakesleeanus*; to my brother, A. P. Meiklejohn, for calling my attention to the possibilities of the method, and to E. M. Crook and D. J. Higgons for supplies of solanine and solanidine.

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The Total Nitrogen Content of Egg Albumin and Other Proteins

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The question of amino-acid analysis became one of increasing importance in this laboratory during 1933, when the isolation of proteins from various forage grasses had been successfully accomplished and evidence for their content of the dietetically essential amino-acids was clearly desirable.

Work has continued without interruption since then, but at times progress has been slow on account of the laborious nature of many of the incidental investigations that we have felt called upon to undertake. During the first two years some preliminary studies of the better-known methods for base and dicarboxylic acid analyses were made by Miller [1935; 1936]; these served to emphasize the large amount of humin formation that attended the hydrolysis of our relatively impure leaf products, and little further progress was made until the origin of this humin had been satisfactorily established by Bailey [1937] and Lugg [1939]. The observation that cystine and, to a lesser extent, tyrosine can condense, like tryptophan, with the furfural derived from pentosan-like impurities during acid hydrolysis to form humin led Lugg, in continuation of work started in Adelaide, to investigate the reliability and limitations of the better-known indirect methods for estimating these three amino-acids, and also methionine [cf. Lugg, 1939]. Meanwhile, Tristram [1939] had explored the applicability to these impure leaf proteins of the Block small-scale gravimetric method of base analysis; progress had been made in an attempt to improve the Foreman method of dicarboxylic acid analysis; and the separation of the monoamino-acids by the Dakin-Fischer methods had been explored. With the experience thus gained it was possible to carry out partial amino-acid analyses of a series of protein preparations from forage crops [cf. Chibnall, 1939].

At this stage in our work we were not specifically interested in obtaining strictly quantitative values for the dicarboxylic acids and bases, for such a refinement was considered unnecessary in view of the somewhat impure nature of our leaf products; all that we had set out to attain was an analysis for each product comparable, from a nutritive point of view, with those already in existence for many seed and animal proteins. During 1936–8 however Bergmann & Niemann reported partial analyses of several proteins, and on the results of these they formulated certain stoichiometric laws governing protein structure. Two of the proteins they had selected for consideration, egg albumin and haemoglobin, had already been submitted to dicarboxylic acid analysis in this laboratory, and our results, which we were fairly certain were still several per cent too low, were higher than theirs and definitely not in keeping with the general conclusions they had drawn. Moreover, in agreement we believe with many others, we were not satisfied with the values they had assigned to the molecular weight of the average amino-acid residue.

In 1938 therefore it was decided to attempt to perfect our analytical methods for the dicarboxylic acids and the bases so as to obtain values which we could feel were reliable to 2% or less. In this we have been as successful as we could reasonably expect, and our investigations, which are still in progress, will be reported in later papers. Meanwhile the bearing that these new results have on the structure of the proteins concerned has been discussed in a recent lecture [Chibnall, 1942], and the interpretation was based in part on a value for the molecular weight of the average amino-acid residue computed from data which include, *inter alia*, the total N of the protein. As it is essential that this latter value be accurately determined we were led to the investigations reported in the present communication.

On reviewing the more recent literature dealing with the preparation and analysis of proteins we found that in many instances the recorded value for the total N of the protein concerned was significantly lower than that accepted by earlier reputable analysts such as Osborne and Sörensen, and the disagreement was especially marked in the case of egg albumin. Now it is common knowledge that the two above-mentioned workers used methods of analysis which they had proved by experience were reliable, and the implication that the work of the later investigators might be untrustworthy needed demonstration, for the position left us not only in doubt as to the value to be used in the computation of the average residue weight, but, as will be discussed later, it shook our confidence in the results of many of the more recent amino-acid analyses of protein hydrolysates and physicochemical studies of proteins in solution.

Osborne invariably determined N on the air-dried sample, as he found that, in general, the anhydrous material was too hygroscopic to be handled without special precautions. For the Kjeldahl analysis he used about 0·1 g. protein, 20 ml. H_2SO_4 and a drop of mercury, the heating being continued for 4–5 hr. after clearing and the oxidation completed by adding a few small crystals of $KMnO_4$ immediately after the gas to the burner had been turned off. Sörensen & Höyrup [1915–17] took for analysis an amount of egg albumin solution corresponding to not more than 20 mg. protein-N and digested this with 20 ml. H_2SO_4, 5 g. K_2SO_4 and 50 mg. Cu wire. The digest cleared in 2–4 hr. and the heating was continued for a further 5 hr. The still boiling liquid was then oxidized by adding cautiously 1·5 g. of dry pulverized $KMnO_4$ in 3 equal lots. Both these protein chemists therefore found from experience that prolonged heating of the digest was necessary for an accurate analysis.

In recent years there has been a natural desire to shorten the time period of digestion and this has led to the introduction of new catalysts such as selenium, its dioxide, or metallic selenates, all of which have been shown to be very effective in promoting the rapid oxidation of many forms of organic-N [cf. Bradstreet, 1940]. These modifications, often applied on a micro-scale, have been shown in many instances to give a satisfactory recovery of N if the heating be continued for less than 1 hr. after clearing, and the notion that the time lag inherent in the older methods had been thereby overcome has no doubt been fostered by the undoubted success that has attended most of the rapid micro-methods of organic analysis introduced by Pregl. When these modified Kjeldahl methods are applied, however, to many materials of biological origin (e.g. wheat, flour, seeds, etc.) there is no absolute check on the results obtained, and in the protein and amino-acid field the uncritical acceptance of their trustworthiness is apparent from the data collected in Table 1 and the discussion which follows.

Table 1. *Nitrogen content of certain proteins, as recorded in the literature*

(All values are calculated on an ash-free and moisture-free basis; those in parentheses indicate the ranges with different samples. K indicates Kjeldahl analysis; D, Dumas analysis.)

Protein	Total N (%)	Reference
Egg albumin, heat coagulated	K 15·55 (15·51–15·59)	Osborne & Campbell [1900]
	D 15·6 (15·57–15·63)	
Egg albumin, heat coagulated	K 15·71 (15·61–15·83)	Vickery & Shore [1932]
Egg albumin, native protein	K 15·60 (15·61–15·59)	Taylor, Adair & Adair [1932]
Egg albumin, heat coagulated	K 15·16	Jones & Moeller [1928]
Egg albumin, heat coagulated	K 15·12 (15·03–15·23)	Calvery [1932]
Egg albumin, native protein	K 15·44 (15·1–15·7)*	Hendrix & Dennis [1938]
Egg albumin, native protein	K 15·36 (15·2–15·6)*	Hendrix & Dennis [1938]
Egg albumin, heat denatured	K 15·27	Bernhardt [1940]
Pepsin, twice recrystallized	K 13·95 (13·8–14·1)	Northrop [1939]
	D 14·8 (14·63–14·83)	
Pepsin	K 15·4	Calvery, Block & Shock [1936]
β-Lactoglobulin, recrystallized	K 15·3†	Palmer [1934]
β-Lactoglobulin, 4 times recrystallized	K 15·14, 15·26‡	Pedersen [1936]
β-Lactoglobulin, thrice recrystallized	? 14·35	Winnick [1942]
β-Lactoglobulin, thrice recrystallized	K 15·6	Cannan [private communication]
β-Lactoglobulin	K 15·6	Sörensen & Palmer [1938]

* Ranges of repeat analyses of 1 sample. † Arnold Gunning method. ‡ Gunning-Pregl micro-method.

Osborne & Campbell [1900] made 4 preparations of what they considered to be pure coagulated egg albumin and obtained concordant values for total N by both the Kjeldahl and Dumas methods (Table 1). Sörensen & Höyrup [1915–17] discussed the precautions necessary to obtain a maximum value by the Kjeldahl method for the protein-N present in an $(NH_4)_2SO_4$ solution of the recrystallized protein, and concluded (p. 181) that 'The factor by which the weight of protein nitrogen has to be multiplied to give the weight of the egg albumin dried over solid potassium hydroxide *in vacuo* at indoor temperature is nearly equal to 6·4 and this does not apply solely to soluble crystallizable egg albumin, but also to denatured egg albumin and eventual middle terms between these two substances'. A factor of 6·4 corresponds to 15·63 % N. More recently Vickery & Shore [1932] have obtained a mean value of 15·71 % N for 3 samples of thrice recrystallized protein, while Taylor, Adair & Adair [1932] record a mean value of 15·60 % N for 2 samples of protein that had been crystallized 7 times. In contrast to these high values, which show reasonable agreement, there are others, much lower, which exhibit considerable irregularities. All these latter appear to have been obtained from samples of recrystallized egg albumin, or of heat-coagulated products derived from them; hence one is led to infer that either the analytical procedures employed must have been faulty or the workers concerned have not realized that the anhydrous products are extremely hygroscopic. Calvery [1932] analysed many preparations of the coagulated protein and recorded a mean value of 15·12 % (Table 1). Rather surprisingly this result is referred to later [Calvery, Block & Shock, 1936] in the following terms: 'It has been previously definitely established that the nitrogen content of crystalline egg albumin when dried at 103–110° and corrected for ash is 15·4 %.' Hendrix & Dennis [1938] made a very large number of analyses of 2 samples of crystalline protein, and also of denatured products obtained from them by the action of dilute alkali, dilute acid and by shaking. Sörensen & Höyrup's technique was employed, and the products were dried to constant weight *in vacuo* at 110° before analysis. The results in each case exhibited considerable irregularities and were submitted to statistical treatment. The values for the native proteins are given in Table 1; those for the alkali-denatured (2 samples), acid-denatured and shaking-denatured products, were 15·21, 15·05, 14·86 and 14·95 % N respectively. Robinson & Hogden [1941] determined the percentage N in 66 samples of heat-coagulated proteins from blood serum by a micro-method using $SeOCl_2$ as a catalyst, the heating being continued for only 20 min. after clearing. Their analyses were made on material dried to constant weight, and the difficulty of dealing with this on account of its hygroscopic nature was admitted; the results ranged from 15·4 to 16·2 % with a mean of 15·78 % N. No control analysis of a standard protein of known N content was recorded. The above citations are by no means exhaustive. A search of the literature covering the last 15 years, the period during which the micro-Kjeldahl method has come more and more into general use, reveals many instances in which investigators have recorded the results of amino-acid analyses in terms of percentage weight of protein, the values being admittedly based on a determination of total hydrolysate-N. In these cases it is rare to find any mention of the mode of preparation or of the purity of the protein used; all too often its N content is not recorded and no indication is given of the procedure by means of which the hydrolysate-N was determined, except perhaps a simple statement that the micro-Kjeldahl method had been employed. The results given below illustrate the magnitude of the errors which may be incurred if insufficient attention be given to what is one of the simplest of analytical procedures.

EXPERIMENTAL

Protein samples analysed

Egg albumin. We have to thank Prof. R. K. Cannan for 2 samples of thrice recrystallized material [Kekwick & Cannan, 1936]. The coagulated protein was prepared in the following way. 160 g. of air-dried material, containing 26·5 % Na_2SO_4, were dissolved in 1·5 l. H_2O and poured slowly, with vigorous stirring, into 15 l. H_2O at 90°. The coagulated protein was filtered off on a Büchner funnel, the mass removed from the paper, stirred up with 15 l. H_2O at 90° and again filtered. The washing was repeated, first with 15 l. H_2O at 90° and then successively with 2–3 l. cold H_2O until the last few filtrates were free from sulphate. The product was next washed with ethanol and ether, dried at 70°, ground to a fine powder and passed through a small-meshed sieve. The final product sampled reproducibly for moisture after storage in a bottle at room temperature for 2 years.

Edestin. This was prepared from freshly ground hemp seed in the usual way and twice recrystallized from hot 5 % NaCl solution [Bailey, 1942].

Amandin. This globulin is of interest as it gives on hydrolysis an unusually high percentage of amide-N. Osborne & Campbell [1896] record a mean value of about 19·3 % N by the Dumas method, but Osborne [1910] in a later review recalled that earlier workers such as Ritthausen found only about 18·7 % and gave it as his opinion that 19·3 % was probably a little too high and suggested 19·0 %. We have to thank Dr Vickery for a sample of this protein prepared from almond seeds in 1915 by Mr Leavenworth. It contained, ash- and moisture-free, 18·66 % N. The product, as was to be expected after the lapse of so many years, left a considerable insoluble residue when extracted with salt solution, and as we were anxious to obtain good crystalline material for various purposes purification was effected in the following way on the advice of Dr K. Bailey.

50 g. protein, 6·25 g. NaCl, 125 ml. H_2O and 6·25 ml. ethanol were ground to a paste in a mortar. *N*/14 NaOH was then added with stirring until the mixture was just blue to thymol blue (glass electrode *p*H 7·8–8·2). The volume was next adjusted to 1250 ml. with H_2O, the mixture brought to 25° and shaken gently without frothing for ½ hr., care being taken to ensure that all sticky particles had been dispersed. The mixture was then filtered 3 times through pulp that had been washed with distilled water. The filtrate was clear, and some insoluble dark brown material was left on the pad. *N*/10 HCl was added to the filtrate with rapid stirring until there was a definite sign of turbidity, and the solution then carefully adjusted to *p*H 6·3. At this stage the precipitate was not bulky and consisted of denatured protein, as shown by adding NaCl to a test sample and gently warming. The mixture was filtered on a well-washed pad, and to the clear filtrate was added with stirring 1/10 vol. of 95% ethanol. A white bulky precipitate appeared, which redissolved on warming to 35°. The clear solution was now cooled rapidly with stirring to 0°, left for 1 hr. at that temperature and the separated crystalline material centrifuged off as rapidly as possible in cooled cups. The paste thus obtained was stirred up with about 3 times its volume of 10% acetone, cooled to 0° and again centrifuged. The product was washed twice with 50% ethanol, 3 times with absolute ethanol, and then 3 times with acetone, being filtered off the last time and sucked free from all extraneous solvent. The final product was dried *in vacuo*. The yield was 18 g.

Haemoglobin. We have to thank Prof. E. J. Cohn for a sample of horse carboxyhaemoglobin prepared in November 1938 by Singher and Morro of the Harvard Medical School. It had been thrice recrystallized, heat coagulated and washed free from chloride. As received the product contained a small amount of fibrous material, undoubtedly filter pulp, which was removed by sifting.

β-Lactoglobulin. We have to thank Prof. R. K. Cannan for two 50 g. samples. The twice recrystallized protein had been coagulated by heat, washed free from inorganic salt and dried through ethanol and ether.

Excelsin. We have to thank Dr K. Bailey for a sample of the recrystallized protein [Astbury, Dickinson & Bailey, 1935].

Casein. This was prepared by the method of Cohn & Hendry [1923], except that the solution was clarified by filtration through paper pulp instead of being passed through a Sharples centrifuge.

Insulin. A crystalline sample was obtained from Boots Pure Drug Company Ltd., Nottingham.

Pepsin. We have to thank Dr J. St L. Philpot for 2 small samples of crystalline material.

Methods of analysis

Moisture and ash contents of proteins. The material for analysis was first finely ground in an agate mortar. For the determination of the moisture content a sample was dried in an oven at 102° to constant weight [Brand & Kassell, 1942]. In general this was attained in 3–5 hr. and the further loss on heating overnight did not exceed 0·1%. The ash content was determined by heating a sample of the air-dried material in a muffle at 550–600° to constant weight.

Kjeldahl incineration. Na selenate was first used as a Kjeldahl catalyst in this laboratory in 1935, and our notebooks show that prior to the present investigation, which was started in 1938, we had made use of a digestion mixture consisting of 20 ml. H_2SO_4, 10 g. K_2SO_4 and 25 mg. Na selenate. In addition 0·65 g. $CuSO_4.5H_2O$ was occasionally added. The recorded results for good recrystallized edestin were 18·4 ± 0·1% N, and for coagulated egg albumin 15·3 ± 0·1% N. When we decided in 1938 to make an amino-acid analysis of these two proteins the difference between our values and those quoted by Osborne came into sharp focus and we realized that our analytical methods needed overhaul. The trouble was finally traced to the short period of digestion (2–3 hr. after clearing) we had been employing and the following procedure was found, after a long trial of many, including those of Osborne, Sörensen, Pregl ($HgSO_4$), and Peters & Van Slyke [1932], to give the most satisfactory results with proteins and protein hydrolysates.

Our experience confirms that of Osborne and many others that oven-dried proteins are too hygroscopic for convenient handling, and we have invariably used air-dried material if sufficient were available for a separate determination of the moisture content. The sample for analysis, which should not contain more than 10–15 mg. N, is weighed out in a small glass flat-bottomed cup, stoppered if there be need to dry to constant weight. After weighing, the cup is dropped carefully into a long-necked 250 ml. Kjeldahl flask in such a way that no protein is scattered; about 5 ml. H_2O followed by 20 ml. N-free H_2SO_4 are added and the mixture boiled gently on the stand until charring commences. The flask is then removed from the stand, cooled slightly and 5 g. catalyst mixture added. The latter is prepared by grinding to a fine powder 80 g. K_2SO_4, 20 g. $CuSO_4.5H_2O$ and 0·34 g. Na selenate. The flask is then returned to the stand and the contents gently boiled. The digest clears in 15–30 min. and the heating is continued fairly vigorously for another 8 hr. or, if convenient, overnight. The cooled digest is finally made up to standard volume and an aliquot withdrawn for NH_3 estimation. It is essential, of course, that control blanks for the complete procedure be carried out at frequent intervals. In the case of protein hydrolysates an aliquot containing about 0·1 mg. N is withdrawn and incinerated in the usual micro-Kjeldahl tube, using 2 ml. acid and 0·5 g. catalyst mixture. The procedure then follows that given above, except that the whole digest is used for the NH_3 estimation.

Van Slyke, Hiller & Dillon [1942] have recently drawn attention to the difficulty of determining the N content of lysine by Kjeldahl analysis. According to our experience low values will always be obtained with this amino-acid unless the digest is boiled much more vigorously than is necessary when dealing with a protein rich in lysine such as lactoglobulin. Using a sample of *dl*-lysine monohydrochloride which gave 15·28% amino-N in 30 min. we have obtained a theoretical recovery of N under our usual conditions provided that the digest is boiled briskly on an electric heater for about 12 hr. Using a gas burner the recovery is irregular (95–100%) and it is advisable to add $HgCl_2$ to the catalyst mixture (32 mg./g.). A theoretical recovery of N can then be obtained in 4–5 hr.; in 2 hr. [cf. Weissman & Schoenheimer, 1941] we obtained 98·5%. We confirm the finding of Vickery (private communication) that histidine requires 8 hr. digestion for a theoretical recovery of N. In our experiments with casein and egg albumin (see Table 2) we obtained 15·3% N for each protein when the digest was heated for no longer than ½ hr. after clearing. Similar results were obtained when using the digestion mixture of Campbell & Hanna [1937].

Lysine (especially) and histidine are no doubt responsible for the much longer heating period required to obtain a maximum recovery of N.

Estimation of ammonia. The usual Pregl micro-distillation apparatus was used. Haack micro-burettes, graduated to 0·05 ml. and reading accurately to 0·01 ml., were used for the *N*/70 NaOH and HCl respectively. The latter was made up in 20% ethanol with Tashiro's indicator according to the directions of Conway & Byrne [1933].

DISCUSSION

A summary showing the reproducibility of our results is given in Table 2. It will be seen that our values for egg albumin are in agreement with those of Vickery & Shore, and slightly exceed those of Osborne and of Sörensen: we find also, in confirmation of Sörensen, that there is no significant difference in the values for the native and the heat-coagulated protein. We would tentatively suggest that the lower and irregular values recorded by Hendrix & Dennis [1938] are due in large part to the fact that they worked with anhydrous material. In our attempts to do this we have invariably found that both the native and heat-coagulated proteins are so hygroscopic that 2–3% of moisture are taken up if the sample is exposed for only a few minutes in a desiccator that has been recently opened to the air of the laboratory; in other words, the anhydrous products have desiccating properties and compete with the desiccant for any water vapour present. Anhydrous edestin does not exhibit this peculiarity to such a marked degree and we note that Hendrix & Dennis's value for this protein (18·70% N) is in excellent agreement with Osborne's and our own. Anhydrous insulin [cf. Miller & du Vigneaud, 1937] and anhydrous pepsin, on the contrary, are extremely hygroscopic and we have found it convenient to use the air-dried material. Although only small amounts of crystalline pepsin were available we have had no difficulty in obtaining comparable results by the Kjeldahl and Dumas methods (cf. Northrop's results given in Table 1). When sending the 2 samples of this protein Dr Philpot informed us that they might contain, as is usual, some non-protein-N; we mention this because a somewhat higher value for total N (15·4%) has been recorded by Calvery, Herriott & Northrop [1936]. Crystalline enzymes and protein hormones are usually isolated in small amount after an extensive procedure, so that the investigator naturally begrudges the material that must be allocated to elementary analysis. We think it timely therefore to issue a warning that such products, when anhydrous, are likely to be extremely hygroscopic and need special precautions in handling. During our investigations of egg albumin we sent samples of the heat-coagulated product to a firm of micro-analysts for determination of the moisture content by drying at 100° *in vacuo*. The results, obtained by what we believe to be a standard procedure, ranged from 6 to 7%, whereas we had ourselves obtained 11% when the product was dried at atmospheric pressure for 4 hr. at 102° in a weighing bottle, the stopper of which was replaced before removal from the oven.

In general, it will be seen that our results confirm the higher values recorded by earlier authorities who used macro-methods. Our value for amandin (18·76%) is in keeping with Osborne's surmise that his own, determined by the Dumas method, was a little too high. In the case of excelsin we had only

Table 2. *Nitrogen content of various proteins*

(Values given in parentheses indicate the ranges of analysis; D indicates Dumas analysis.)

Protein preparation	Air-dried material: Moisture content %	Air-dried material: Ash content %	No. of determinations	N, moisture- and ash-free, %
Egg albumin, native protein A	13·3	26·5	8	15·76 (15·69–15·80)
Egg albumin, heat coagulated A	11·0	0·44	7	15·76 (15·71–15·79)
			6	D 15·72 (15·69–15·84)
Egg albumin, heat coagulated B	9·8	0·5	7	15·74 (15·67–15·81)
Edestin, Sample A	11·2	0·05	7	18·65 (18·62–18·70)
Edestin, Sample B	11·1	0·2	6	18·70 (18·66–18·76)
β-Lactoglobulin	6·7	0·28	6	15·58 (15·53–15·67)
Casein	10·2	0·75	8	15·73 (15·72–15·75)
Excelsin	8·4	0·1	4	17·94 (17·84–18·02)
Amandin, Sample A	9·3	0·1	2	18·77 (18·76, 18·78)
Amandin, Sample B	8·1	0·94	2	18·75 (18·71, 18·78)
Carboxyhaemoglobin (horse), Sample A	12·7	—	2	16·80* (16·82, 16·78)
Insulin	10·0	1·24	2	15·54 (15·53, 15·54)
Pepsin, Sample A	10·87	1·2	1	14·95
			1	D 14·7
Pepsin, Sample B	9·94	1·2	1	14·78
			1	D 14·83

* Ash not deducted.

a small amount available and it was necessary to analyse the anhydrous material. The values obtained show wider variations than we expect when using air-dried material and we see no reason to doubt the slightly higher value (18·17 %) reported by Osborne & Harris [1905] for a sample of good crystalline material. Taken as a whole our results demonstrate that in recent years many investigators, whose interests have naturally been focused on the main aspects of their research, have tended to regard the determination of protein-N as a simple matter demanding no special precautions and have paid insufficient attention to the technique of Kjeldahl analysis. These findings are disturbing, for it is becoming more and more customary in amino-acid analysis, especially when only small amounts of protein are available, for the investigator to employ what he regards as moisture-free material, or alternatively, to take an unknown amount of the air-dried product and compute the weight of moisture-free and ash-free protein from a determination of total N in the hydrolysate, using as a conversion factor Osborne's or some other value for the N content of the protein. The latter procedure is clearly at the mercy of the Kjeldahl analysis, and if the technique employed leads to a low result the computed weight of protein is in error, as must also be the results of all subsequent amino-acid estimations. A similar criticism will apply to physico-chemical studies of proteins in solution, wherein the amount of protein present is computed from a Kjeldahl analysis of an aliquot.

SUMMARY

1. Attention is drawn to the fact that in recent years many protein chemists have been employing a technique for the Kjeldahl estimation of N in proteins and protein hydrolysates which gives low results; this is especially true if micro-methods have been used. Instances are cited in which recorded values for the N content of proteins are significantly lower than those obtained by earlier authorities such as Osborne & Sörensen.

2. It is shown that these low values are due to two causes: (1) The digestion period has been too short; with proteins and protein hydrolysates it is necessary to continue the heating for 8 hr. or more after the digest has cleared. (2) Certain proteins (e.g. egg albumin, pepsin and insulin), when anhydrous, are so hygroscopic that they cannot be handled without special precautions; moisture and N contents therefore should be determined, whenever possible, on separate samples of air-dried material.

3. Using the technique described the following values have been obtained for the N content of the moisture- and ash-free protein: egg albumin (native and coagulated), 15·76 %; edestin, 18·7 %; β-lactoglobulin, 15·58 %; casein, 15·73 %; amandin, 18·75 %; insulin 15·54 %; horse carboxyhaemoglobin (moisture- but not ash-free), 16·8 %.

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