XLIV. THE PROTEINS OF GRASSES.

III. THE CYSTINE CONTENT OF CERTAIN GRASSES AND OTHER PASTURE PLANT PROTEINS.

BY ALFRED POLLARD AND ALBERT CHARLES CHIBNALL.

*From the Biochemical Department, Imperial College of Science and
Technology, South Kensington, London.*

(Received January 8th, 1934.)

A FEW years ago Robertson [1928] suggested that since the keratin of wool contained such a high proportion of cystine (about 13 %) the carrying capacity of any country for sheep which are grown for wool production might well be determined by the capacity of its pasture plants to produce cystine. Attention thus became focused on the cystine content of grasses and other pasture plants. Now the major part of this cystine will presumably be present as a constituent of the leaf proteins, and to determine this protein-cystine it is necessary to hydrolyse the protein with strong acid or (less satisfactorily) to digest it with appropriate proteolytic enzymes. As no method for preparing these proteins has hitherto been available earlier workers have found it necessary to submit the whole grass or pasturage itself to acid hydrolysis or to enzymic digestion. This means, in effect, that the proteins in the leaf material have been treated in the presence of from three to six times their own weight of cellulose, sugars, organic acids *etc.*, and it is not at all surprising that under these very unsatisfactory conditions the highly specific colour reaction of Sullivan for cystine was found difficult to carry out and indicated the presence of only a trace of this amino-acid [Aitken, 1930; Evans, 1931; Henrici, 1932]. Evans [1931] also, assuming the alkali-labile sulphur to represent two-thirds of the cystine-sulphur, found that the various samples of pasturage examined by him might contain from 0.07 to 0.18 % of cystine on the basis of dry matter. He pointed out however that not all the labile sulphur of proteins can correctly be ascribed to cystine and suggested that the true cystine content of grass must be less than 0.1 % of the dry matter.

Rimington and Bekker [1932], adopting the figure 0.01 % of dry matter as a fair average value for the cystine content of grass, calculate that a sheep producing a 12 lb. fleece, which contains about 0.78 lb. of cystine, would have to eat 21.4 lbs. of dry grass daily in order to obtain this amount of preformed cystine. Since sheep under optimum conditions will consume only 4 lbs. of dry matter daily they conclude that grasses are deficient in cystine. Woodman and Evans [1932] disagree with this conclusion, and point out that Evans's value, 0.1 % of the dry matter, for the cystine content of cultivated English pasturage would enable sheep to secure the cystine necessary for a 12 lb. fleece during a grazing period of only 195 days. Rimington *et al.* [1933] maintain, however, that the value of 0.1 % for the cystine is too high, and in support of their contention instance a stud ram which produced a fleece of 46 lbs. on a diet consisting almost entirely of lucerne.

These discussions were based on inadequate experimental evidence. During the past three years methods have been perfected in this laboratory for pre-

paring the proteins of grasses and other forage plants, and we have now completed a fairly searching enquiry into their cystine content. We first determined cystine by the well-known methods of Sullivan. The protein hydrolysates were, however, deeply coloured and could not be satisfactorily decolorised by means of norite or kaolin without large losses of cystine. Our results with cocksfoot protein, which we used exclusively in our preliminary experiments, suggested only 0.1–0.2 % of cystine.

The method of Vickery and White [1933], however, details of which were kindly supplied to us some months before publication by Dr Vickery, gave very much greater values, 1.1–1.2 %. According to this method the protein is hydrolysed in the presence of tin, which reduces the cystine to cysteine as it is set free. The cysteine is then precipitated from solution under appropriate conditions as the very insoluble cuprous mercaptide. This precipitate is decomposed with hydrogen sulphide, the cuprous sulphide removed and the organic sulphur in the clarified solution determined. In all the cases examined by them the cystine content of the protein calculated from this sulphur value agreed reasonably well with the content determined by Sullivan's procedure. In the case of our leaf proteins, as mentioned above, there was a great discrepancy. Moreover the value for cystine determined by Sullivan's method on the clear filtrate from the cuprous sulphide was similar to that obtained on direct hydrolysis. Clearly the Vickery and White procedure was estimating, in addition to the sulphur of cystine, the sulphur of (1) an unknown amino-acid giving an insoluble cuprous salt, or (2) products arising from the decomposition of cystine during the acid hydrolysis of the protein in the presence of the small amount of impurity which all our grass protein preparations contain.

We have been able to eliminate the latter alternative by making use of the new modification of Sullivan's method introduced by Prunty [1933]. The cystine in the hydrolysate is reduced to cysteine by means of zinc in acid solution instead of with sodium cyanide, and during the reduction the hydrolysate is almost completely decolorised. Accurate and easily reproducible values for the cystine content of the leaf proteins were readily obtained. Added cystine could also be recovered in 90–95 % yield, showing that no appreciable destruction of cystine was taking place during hydrolysis. Additional proof was afforded by experiments in which the protein was hydrolysed by enzymes.

One very interesting result of our research has therefore been the clear proof that leaf proteins must contain at least one unknown sulphur-containing amino-acid, because Vickery and White have shown that methionine, which is present in these proteins, is not precipitated under their conditions as a cuprous salt. This unknown amino-acid yields part of its sulphur as sulphide on alkaline hydrolysis and may quite likely be the same product which the experiments of Zahnd and Clarke [1933] show to be present in egg-white.

The cystine content of the pasture leaf proteins, determined by the Prunty-Sullivan colorimetric method, varies between 0.25 and 1.25 %, as shown in Table I, and by calculation it is possible to show quite definitely that a mixed herbage of the type which constitutes the pasturage of sheep in this country contains an adequate amount of cystine for the production of a 12 lb. fleece. Lucerne protein has also been examined, and as it is relatively rich in cystine there is no reason to doubt that the prize ram referred to by Rimington *et al.* [1933] also obtained all the necessary cystine from its diet. As the sulphur of the unknown amino-acid referred to above is not stable to alkali it is clear that Evans's determinations of labile sulphur in English pasture grasses were not a valid basis for calculating their cystine contents.

EXPERIMENTAL.

Preparation of the proteins. Full details have been given in a previous paper [Chibnall *et al.*, 1933]. The nitrogen content of the various preparations ranged from 10 to 15 %, owing to the presence of non-nitrogenous impurities whose chemical composition is not yet clearly understood [Miller and Chibnall, 1932]. The percentage of cystine determined on these preparations therefore falls short of the percentage in the protein by an amount depending on the proportion of these impurities present. Leaf proteins known to be pure contain from 15.7 to 16.7 % N [Chibnall and Grover, 1926]. On the assumption that the grass and other pasture plant proteins should also contain 16 % N we have, in the Tables given below, calculated the percentage of cystine in the protein on this basis. Adoption of this figure has the additional advantage that in our subsequent discussion on the cystine content of pasture in relation to wool production we are able to make direct comparison with the conventional protein ($N \times 6.25$) of the agricultural chemist.

Colorimetric estimation of cystine by the Prunty modification of Sullivan's method. The protein (0.5–1.0 g.) was heated with 25 ml. of 20 % hydrochloric acid in a flask immersed in a boiling water-bath for 2 hours, and then gently boiled under reflux overnight. The flask was cooled in ice water, 40 % aqueous sodium hydroxide added to p_H 2.5 and the precipitated humin separated from the yellow or orange solution by centrifuging. To the humin remaining in the centrifuge-tube 5–10 ml. of dilute sodium chloride solution previously adjusted to p_H 2.5 were added, and the tube was immersed in a boiling water-bath for 10 minutes, the mixture being frequently stirred. The humin was again centrifuged off and the washing repeated at least eight times in all. Further washings gave no appreciable amount of cystine by the colorimetric method. The use of aqueous sodium chloride instead of water for washing the humin was necessary to prevent the formation of colloidal suspensions. The final solution was brought to a convenient volume, usually 100 ml., the last few washings being concentrated, if necessary, *in vacuo*.

Two portions of 5 ml. were measured into centrifuge-tubes, and to each was added 1 drop of concentrated hydrochloric acid and a few mg. of zinc dust. The tubes were then heated in a boiling water-bath for 5 minutes, cooled and centrifuged. The faintly yellow solutions were decanted into 15 ml. standard flasks, containing 1 ml. of 25 % aqueous sodium hydroxide and 2 ml. of 10 % aqueous sodium cyanide, the tubes being washed out with the requisite amount of water to make up the standard volume. At the same time a convenient amount of cystine was reduced and treated in a similar way. To one of the unknown solutions (A) and to the known cystine solution (S) were added 1 ml. of a 1 % solution of sodium β -naphthoquinonesulphonate and then immediately 5 ml. of a 10 % solution of sodium sulphite in 0.5 *N* aqueous sodium hydroxide; while to the other unknown (B) was added only the sulphite solution. After remaining at 25° for 30 minutes to each of the three solutions was added 1 ml. of a 2 % solution of sodium hydrosulphite in 0.5 *N* aqueous sodium hydroxide. The colour of a known depth of solution (A) was then compared with the colour of the same depth of the blank solution (B), superimposed on a variable depth of the standard solution (S), using a Klett colorimeter. No rapid fading of the red colour has been observed with our hydrolysates such as that recorded by Prunty in the case of gelatin. After standing in the air for half an hour the solutions become slightly darker, but the addition of a very small amount of sodium hydrosulphite at once restores the original colour. The results for the pasture leaf proteins are fully set out in Table I.

Table I. *The cystine content of pasture leaf proteins estimated by the Prunty modification of Sullivan's method.*

Species	Sample of leaf protein		True protein (N = 16 %) Cystine %
	Total N (ash-free) %	Cystine %	
Red clover (<i>Trifolium pratense</i>)	12.6	0.16	0.21
Bent (<i>Agrostis</i> sp.)	10.3	0.15	0.24
Cocksfoot (<i>Dactylis glomerata</i>) (AB)	14.6	0.26	0.28
" (AC)	13.4	0.20	0.24
" (AD)	13.2	0.20	0.24
" (AG)	13.2	0.21	0.26
" (AH)	13.5	0.23	0.27
Rough-stalked meadow grass (<i>Poa trivialis</i>)	13.8	0.23	0.27
Wild white clover (<i>Trifolium repens</i>)	13.2	0.26	0.32
Timothy (<i>Phleum pratense</i>)	13.8	0.29	0.34
Perennial ryegrass (<i>Lolium perenne</i>)	12.7	0.38	0.48
Yorkshire fog (<i>Holcus lanatus</i>)	11.8	0.36	0.49
Italian rye-grass (<i>Lolium italicum</i>)	14.0	0.59	0.67
Tall fescue (<i>Festuca elatior</i>)	13.6	0.60	0.70
Lucerne (<i>Medicago sativa</i>) (A)	14.4	0.66	0.73
Crested dog's tail (<i>Cynosurus cristatus</i>)	14.9	0.70	0.75
Meadow fescue (<i>Festuca pratensis</i>)	13.9	0.67	0.80
Red fescue (<i>Festuca rubra</i>)	14.2	0.77	0.87
Hard fescue (<i>Festuca duriuscula</i>)	14.6	0.85	0.90
Chewings fescue (<i>Festuca rubra</i> var. <i>Fallax</i> (Hack.))	14.0	0.85	0.93
Lucerne (<i>Medicago sativa</i>) (B)	15.8	1.20	1.20

Possible losses in the colorimetric estimation of cystine. The possibility that the impurities present in our protein preparations were causing extensive breakdown of cystine was investigated by adding a known amount of this amino-acid before hydrolysis. The results of two typical experiments with cocksfoot and crested dog's tail proteins are given in Table II, and show a recovery of added

Table II. *Showing the recovery of added cystine.*

Protein preparation	Cystine %	Cystine added %	Total cystine estimated %	Recovery of added cystine %
Standard HCl hydrolysis (Prunty):				
Cocksfoot (AC)	0.19	0.15	0.31	91
"	0.19	0.50	0.65	94
Crested dog's tail	0.70	0.70	1.36	97
"	0.70	0.70	1.34	95
Hydrolysed with H ₂ SO ₄ in presence of tin:				
Cocksfoot (AB)	0.25	—	0.21	84
"	0.25	0.63	0.74	84

cystine in each case of over 90 %. Lugg [1933] has shown that cysteine, produced from cystine by heating with stannous chloride and hydrochloric acid, may suffer almost complete decomposition if hydrolysed in the presence of relatively large amounts of sugars and amino-acids. With pure proteins Vickery and White [1933] experienced no such loss. As hydrolysis with hydrochloric acid in the absence of oxygen [Okuda, 1926] showed that cocksfoot protein contained about 0.1 % of cysteine we think it worth while to record the following experiment.

Two portions of 1 g. of cocksfoot protein (AB) were taken, and to one were added 6.3 mg. of cystine. Each portion was then hydrolysed with 20 ml. of 8N H₂SO₄ for 20 hours in the presence of 1 g. of granulated tin. The solutions were

cooled, 40 % aqueous sodium hydroxide was added to p_H 2.5 and the mixtures, each containing the humin in suspension, were saturated with hydrogen sulphide. The resulting pale yellow solutions were cleared by centrifuging, and the residue of stannous sulphide and humin was washed eight times successively with dilute hydrochloric acid. In neither case did the last two washings give any nitroprusside reaction. The solutions were concentrated *in vacuo* and the cystine + cysteine present estimated colorimetrically in the usual way. The results are given in Table II. If it be assumed that the cystine content of the protein be that given on standard hydrochloric acid hydrolysis, then the amount of cystine estimated in each case amounted to 84 %. The loss is possibly due in part to the fact that air oxidation of cysteine to cystine (which must have taken place to a small extent during the concentration *in vacuo*) is not strictly quantitative, but we think that the major loss is due to adsorption of cysteine on the stannous sulphide; even greater losses (20–30 %) were found by Lugg [1933] when cystine was heated with stannous chloride and hydrochloric acid, the metal being subsequently removed as sulphide. The results show fairly conclusively that Prunty's modification of the Sullivan method estimates 90–95 % of the total cystine and cysteine present in the pasture leaf proteins.

The alkali-labile sulphur as a measure of cystine content. The procedure employed by Osborne [1902] was followed. The results were somewhat erratic, as Osborne found with seed proteins, but representative values for the alkali-labile sulphur of cocksfoot protein are given in Table III. If we accept Osborne's conclusion that this represents about two-thirds of the total cystine-sulphur, the value for cystine in each case would be approximately three times that given by the Prunty-Sullivan method. It appears from this result that a determination of alkali-labile sulphur estimates not only the sulphur of cystine but also in large part the sulphur of the unknown sulphur-containing amino-acid whose presence in the hydrolysis products of these leaf proteins is clearly demonstrated by the Vickery and White procedure outlined below. It follows therefore that the cystine values for pasture grasses quoted by Woodman and Evans [1932], which are based on determinations of alkali-labile sulphur by Evans [1931], are not valid.

Table III. *Showing the distribution of organic sulphur in certain pasture leaf proteins.*

Species	Protein preparation Total N (ash-free) %	— True protein (N = 16 %)				Alkali- labile sulphur %	Total sulphur %
		Cystine- sulphur (Sp) (Prunty- Sullivan)	Cystine- sulphur (Sv) (Vickery and White)	Sv - Sp			
		%	%				
Cocksfoot (AB)	14.6	0.072	0.26	0.19	0.12	1.10	
„ (AC)	13.4	0.061	0.31	0.25	0.15	1.30	
„ (AG)	13.2	0.063	0.31	0.25	—	1.40	
Tall fescue	13.6	0.184	0.29	0.11	—	1.21	
Red fescue	14.2	0.221	0.42	0.20	—	1.56	
Hard fescue	14.6	0.24	0.38	0.14	—	1.85	
Perennial ryegrass	12.7	0.125	0.26	0.14	—	1.56	
Lucerne	14.4	0.19	0.28	0.09	—	1.55	

Estimation of cystine(mercaptide)-sulphur by the method of Vickery and White [1933]. 5–10 g. of protein were hydrolysed in the presence of tin and the cysteine precipitated as the cuprous mercaptide. The procedure of these authors gave

consistent results without difficulty, and, as will be seen from Table III, the values for cystine(mercaptide)-sulphur are in all cases greatly in excess of those obtained by the Prunty-Sullivan colorimetric method. If the latter represent the true cystine-sulphur, as we believe, then it follows that the cuprous oxide must have precipitated at least one other sulphur-containing amino-acid. Confirmation of the low cystine content of the cuprous precipitate was obtained in the following experiment.

46 g. of hard fescue protein, containing 0.84 % of cystine by the Prunty-Sullivan colorimetric method, were treated according to the procedure of Vickery and White. The cuprous mercaptide was then decomposed with hydrogen sulphide in the presence of sulphuric (not hydrochloric) acid. The cuprous sulphide was centrifuged off and washed repeatedly by boiling with dilute sulphuric acid until the washings gave no nitroprusside reaction for cysteine. The clean solution and washings were united, adjusted to p_H 7 with barium hydroxide and aerated for 1 hour, after which time no free cysteine could be detected by the nitroprusside reaction. The solution was then made slightly acid with sulphuric acid and concentrated *in vacuo*. The precipitate of barium sulphate was centrifuged off and washed repeatedly with 0.002 *N* sulphuric acid. The solution and washings were united and concentrated *in vacuo* to 250 ml. Cystine, by the Prunty-Sullivan method, amounted to 0.27 g., equivalent to 0.6 % of the protein taken for analysis. Vickery and White show that a certain amount of cysteine destruction occurs during the decomposition of the cuprous mercaptide. This amount of cystine contains 0.072 g. of sulphur, whereas the total organic sulphur present was 0.173 g.

The solution was then concentrated *in vacuo* to 25 ml. and an equal volume of alcohol added. The pale brown precipitate which came out in the course of a few days was filtered off, dissolved in a few ml. of dilute hydrochloric acid and a small residue of barium sulphate filtered off. The clear filtrate was then cooled to 0° and saturated with hydrogen chloride gas. The mass of fine needle-shaped crystals, typical of cystine dihydrochloride, which separated overnight, was filtered off and washed with a little cold absolute alcohol. The weight was 0.14 g., and the mother-liquor on treatment gave a further 0.06 g., making 0.22 g. in all, a yield of 60 % of that estimated colorimetrically. For analysis (Schoeller) the material was dried at 60° *in vacuo* over phosphorus pentoxide. (Found: S, 20.6, 20.4 %. $C_6H_{14}O_4N_2S_2Cl_2$ requires: S, 20.45 %.) The yield of cystine compares favourably with that of Vickery and White, if it be remembered that our solutions contained nearly an equal amount of another sulphur-containing amino-acid.

The liberation of cystine during enzymic digestion. Additional evidence that the Prunty procedure gives a true estimate of the cystine content of the proteins, and that the Vickery and White procedure is not estimating any decomposition products of cystine, was obtained from experiments in which the protein was broken down by enzymic digestion and was therefore not subject to the action of products set free from the non-protein impurities by the strong acid hydrolysis. Six digests of cocksfoot protein and one of Italian ryegrass protein have been examined; we shall however only quote one result of the former. Details of the experiments follow closely those concerned with the amide-N of proteins [Damodaran, 1932, Damodaran *et al.*, 1932], the enzymes used being pepsin, trypsin and intestinal (pig) erepsin in succession.

The following account concerns the digestion of cocksfoot protein (AB). (Data corrected for added enzyme preparation.) The protein (9.5 g.) was suspended in 400 ml. of dilute hydrochloric acid (p_H 1.9) containing 0.4 g. of pepsin.

The protein rapidly settled, and the flask was gently shaken at intervals during 41 hours. At the end of that time much of the protein still remained undissolved, the supernatant liquid containing only 16.6 % of the total N. Previous digests had shown that no appreciable amount of cystine was liberated by pepsin [cf. Jones and Gersdorff, 1933]. *N* sodium hydroxide solution was then added to p_H 8.2, when the major part of the protein residue was at once dissolved. 0.4 g. of trypsin was next added, together with 0.5 % of tricresol as antiseptic. It was necessary to adjust the digest to p_H 8.2 every few hours. Cystine and amino-N were determined at intervals, as shown in Table IV. At the end of 72 hours' digestion, when no appreciable protein residue remained, 40 ml. of pig erepsin were added. The solution was maintained at p_H 8.0, and the amino-N reached a maximum at the end of a further 17 hours. In the case of cocksfoot no appreciable increase of cystine was observed with erepsin, though a definite increase was noted in the case of Italian ryegrass. All the enzymic digests were very deeply coloured, and on neutralisation to about p_H 2.5 a large amount of material, partly humin and partly of a proteose nature, was thrown down and was difficult to wash. The cystine determinations were therefore not as consistent or as reliable as those obtained with corresponding acid hydrolysates. It was observed in each case that if the digestion with either trypsin or erepsin were prolonged the cystine value fell slowly from the maximum, indicating a slow secondary decomposition of cystine under the conditions of the experiments.

The results however leave no doubt that the amount of free cystine liberated was of the magnitude that one would expect if the total cystine in the protein were that given by the Prunty-Sullivan colorimetric method and not that given by the Vickery and White mercaptide method; in the case of the cocksfoot protein quoted in Table IV the liberation of cystine was 70 % and of amino-N was 58 % of the maximum given on acid hydrolysis, while with the Italian ryegrass the corresponding figures were 62 % and 64 % respectively.

Table IV. *Showing the amount of cystine set free during the enzymic digestion of cocksfoot and Italian ryegrass proteins.*

Enzyme	Duration of digestion (hours)	Cocksfoot protein (AB) 14.6 % N 0.25 % cystine		Italian ryegrass protein 14.0 % N 0.58 % cystine	
		Cystine (% total protein)	Amino-N (% total N)	Cystine (% total protein)	Amino-N (% total protein N)
Pepsin	41	—	2.4	—	—
Trypsin	24	0.09	—	0.20	—
"	48	0.10	—	0.24	—
"	72	0.18	28	0.30	34.8
Erepsin	17	0.17	40.2	0.36	44.8
"	41	0.15	40.2	0.33	45.0

The total organic sulphur of the proteins. The experimental data given above show that the hydrolysis products of all the pasture leaf proteins examined contain cystine, and that this can be readily estimated by the highly specific colorimetric method of Sullivan. They show also that another amino-acid, probably one containing a sulphydryl group, must also be present. It will be seen from Table III, however, that these two amino-acids, both of which are estimated by the method of Vickery and White, account for only about one-quarter of the organic sulphur present in the proteins. An estimation of methionine on cocksfoot protein by the indirect method of Baernstein [1932]

suggests that the remainder may be due to this amino-acid. Further investigations into the constitution of the unknown amino-acid and of the methionine content of the proteins are in progress.

Reference to Table II shows that in the case of cocksfoot protein with a low cystine content the Prunty-Sullivan method estimates only 90–95 % of the total amount present. If the protein preparation were very impure, such as that of bent or yarrow (N=10 %), it is probable that the loss could be even greater. When preparing the data for Table V, therefore, we have in these cases made what we consider a legitimate allowance for a possible loss during the estimation of from 5–15 % of the total cystine. We have in a few instances determined the cystine content of the dried plant material itself by the Prunty-Sullivan procedure described above. After the reduction process the solutions are somewhat darker than in the case of the proteins, but the red colour subsequently developed by the cysteine can be readily estimated. Low values were always obtained, and added cystine was destroyed to the extent of 40–50 %. This result was not unexpected.

The cystine content of pastures and wool production. Table V gives the calculated amount of cystine, in terms of total dry matter, present in the various

Table V. *Cystine content of pasture plants at various protein levels.*

Species	Percentage of cystine in protein (N=16 %)	Cystine as a percentage of total dry matter in the herbage, containing					
		Protein 8 %	Protein 12 %	Protein 16 %	Protein 20 %	Protein 24 %	Protein 28 %
Yarrow, red clover	0.25	0.02	0.03	0.04	0.05	0.06	0.07
Bent, cocksfoot, rough stalked meadow grass	0.30	0.024	0.036	0.048	0.06	0.072	0.084
Timothy, wild white clover	0.35	0.028	0.042	0.056	0.07	0.084	0.098
Perennial ryegrass, Yorkshire fog	0.50	0.04	0.06	0.08	0.10	0.12	0.14
Italian ryegrass, tall fescue	0.70	0.056	0.084	0.112	0.14	0.168	0.196
Lucerne (A)	0.75	0.06	0.09	0.12	0.15	0.18	0.21
Crested dog's tail, meadow fescue	0.80	0.064	0.096	0.128	0.16	0.192	0.224
Red fescue, hard fescue	0.90	0.072	0.108	0.144	0.18	0.216	0.252
Chewings fescue	0.95	0.076	0.114	0.152	0.19	0.228	0.266
Lucerne (B)	1.2	0.096	0.144	0.192	0.24	0.288	0.336

pasture plants at certain definite protein levels. These values are based on the assumption that the protein is the sole source of cystine. They must therefore be regarded only as minimum values, because cystine or cystine peptides may possibly be present in the water-soluble, non-protein products of the plants, and these have not yet been investigated.

Before attempting to assess the value of these plants as diet for sheep it is necessary to make one or two observations concerning the actual samples which were used for preparing the proteins. All the grasses were taken from plots which had been cut at least once a fortnight, and the samples consisted almost entirely of blades. The red clover and lucerne (A) were each cut about 1 inch above ground, and both samples consisted of somewhat stunted plants with relatively few leaflets. The protein preparations therefore consist of leaf protein well adulterated with that from the stems. The wild white clover and lucerne (B),

on the contrary, were from luxuriant crops, and both were cut with a scythe held well above the ground so that the sample consisted chiefly of leaflets obtained from the upper few inches of the plants. The protein preparations, therefore, are very rich in the leaf protein. This accounts for the striking difference between the two samples of lucerne protein. Sample A, which contained 14.4 % N and 0.73 % cystine, was prepared from lucerne containing 17.2 % true protein. Sample B, which contained 15.8 % N and 1.2 % cystine, was prepared from lucerne grown in Connecticut, U.S.A., containing about 28 % true protein [Chibnall and Nolan, 1924].

With regard to the cystine content of pastures and wool production the data given in Table V can only be discussed in general terms because in actual practice the herbage is always mixed and may change considerably in botanical composition during the season. Furthermore, the question of the amount of ingested cystine which is catabolised or excreted is outside the scope of this paper, so that in the following discussion we can do no more than attempt to show that the minimum amount of cystine ingested is greater than that required for wool-protein synthesis.

If we assume that a sheep eats pasturage containing 4 lbs. of dry matter daily, then the 0.39 lb. of cystine necessary for an average British fleece of 6 lbs. would be secured during one year on a diet containing only 0.0265 % of cystine on the basis of dry matter. This is well below the amount which would be provided by the poorest of pasture consisting chiefly of bent, even if we ignore the fact that such pastures would generally contain a small proportion of one of the fescues, which are rich in cystine. The winter grazing (8 % protein) might be on the borderline, but for 6-8 months during the growth period the protein level would exceed 12 %, and the cystine content of the diet would be in excess of 0.04 %. A 12 lb. fleece, which is the weight discussed by Rimington and Bekker [1932], contains 0.78 lb. of cystine, and for this intake the sheep would require a diet throughout the year containing 0.053 % of cystine on the basis of dry matter. In Great Britain a cultivated pasture of the type which might be expected to carry sheep bearing such a fleece would probably consist in large part of perennial ryegrass, with subordinate amounts of Italian ryegrass, rough-stalked meadow grass, wild white clover and bent at the appropriate season. From March to October the pasture would contain on an average nearly 20 % of protein on a basis of dry matter, so that the cystine content of the diet throughout this period would vary between about 0.08 and 0.1 %. This grazing period alone therefore would provide more than enough cystine for the fleece. During the winter months the protein content of the grazing would fall, but the early appearance of Italian ryegrass would help materially in maintaining an average cystine content in the diet of about 0.05-0.06 %. From an agricultural point of view these descriptions of poor and good quality pastures are necessarily very incomplete, and they are used here only as simplified examples to illustrate our general contention that the pastures of Great Britain almost certainly contain considerably more cystine than the sheep, which they normally carry, require for wool-protein synthesis, especially if it be remembered that sheep will always eat preferentially the young tips of the leaves, which are the richest in protein and therefore in cystine.

In many countries lucerne meal forms one of the principal winter rations of sheep. This has a protein content of about 15 % and is made from cuttings of the whole plant, corresponding to lucerne (A) in Table V. It would therefore have a cystine content of about 0.12 %. If it contained proportionately more leaf material than our sample, which is very probable, then the cystine content

at this protein level would approach the value 0.19 % for lucerne (B) in Table V. These figures are very high indeed when compared with the minimum intake required for the 6 and 12 lb. fleeces mentioned above.

The South African stud ram referred to by Rimington *et al.* [1933], which clipped a record fleece of 46 lbs., was stated to have been fed almost entirely on lucerne. Such a fleece would contain about 3 lbs. of cystine, and on a diet containing 4 lbs. of dry matter daily this amount of cystine would be consumed in one year if the lucerne contained 0.21 % of cystine. This is higher than the value one would expect from ordinary lucerne meal, but a prize stud animal of this type would undoubtedly be given the best possible diet. During the appropriate season it would be turned out to feed on fresh lucerne, and it would undoubtedly choose in preference the leafy lucerne tops. One of us (A. C. C.) analysed many samples of such material collected during the summer of 1924 from crops growing around New Haven, Connecticut, U.S.A., and the true protein content in all cases was never less than 24 % and generally 30 % of the total dry weight. Such material would contain from 0.25–0.3 % of cystine. Lucerne leaf-meal, containing 20–24 % of protein, and hence 0.24–0.27 % cystine, might have formed part of the diet in the stall. In the absence of precise data on the ram's diet, therefore, a cystine intake more than sufficient even for a 46 lb. fleece does not appear extravagant.

SUMMARY.

The cystine content of several typical grass and other pasture plant proteins has been determined by Prunty's modification of Sullivan's colorimetric method. The grass proteins all contain cystine, in amount varying from 0.3 to 0.95 %. Lucerne leaf protein is particularly rich in cystine and contains 1.2 %.

The gravimetric method of Vickery and White gives in all cases a much higher value for cystine-sulphur than the amount shown to be present and must include the sulphur of an unknown amino-acid.

The protein-cystine content of the grasses and other pasture plants at various protein levels has been calculated, and it appears that pasturage contains ample cystine for the wool-protein requirements of the sheep which it normally carries.

We should like to record our thanks to the Imperial Chemical Industries, Ltd., for a grant to cover the cost of this research, to their staff at Jealott's Hill for many helpful discussions and to Mr R. G. Westall for analytical assistance.

REFERENCES.

- Aitken (1930). *Biochem. J.* **24**, 250.
- Baernstein (1932). *J. Biol. Chem.* **97**, 663.
- Chibnall and Grover (1926). *Biochem. J.* **20**, 108.
- Miller, Hall and Westall (1933). *Biochem. J.* **27**, 1879.
- and Nolan (1924). *J. Biol. Chem.* **62**, 173.
- Damodaran (1932). *Biochem. J.* **26**, 235.
- Jaaback and Chibnall (1932). *Biochem. J.* **26**, 1704.
- Evans (1931). *J. Agric. Sci.* **21**, 806.
- Henrici (1932). *Eighteenth Report, Dir. of Vet. Services and Animal Ind.* Pretoria, August 1932, p. 579.
- Jones and Gersdorff (1933). *J. Biol. Chem.* **101**, 657.

- Lugg (1933). *Biochem. J.* **27**, 1022.
Miller and Chibnall (1932). *Biochem. J.* **26**, 392.
Okuda (1926). *Proc. Imp. Acad. Tokyo*, **2**, 277.
Osborne (1902). *J. Amer. Chem. Soc.* **24**, 140.
Prunty (1933). *Biochem. J.* **27**, 387.
Rimington and Bekker (1932). *Nature*, **129**, 687.
—— — and Kellermann (1933). *Nature*, **132**, 63.
Robertson (1928). *Coun. Sci. Ind. Res. Australia, Bull.* No. **39**.
Vickery and White (1933). *J. Biol. Chem.* **99**, 701.
Woodman and Evans (1932). *Nature*, **130**, 1001.
Zahnd and Clarke (1933). *J. Biol. Chem.* **102**, 171.