THE COMPOSITION OF FORAGE CROPS.

A thesis submitted to the University of Glasgow for the Degree of Doctor of Philosophy in the Faculty of Science.

By

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September, 1948.

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ACKNOWLEDGMENT.

Grateful thanks are due to the Council of the Institute for providing the facilities in carrying out these experiments; to Dr. N.C. Wright and Dr. J.A.B. Smith for their interest and encouragement; to Dr. R. Waite for his very stimulating advice and criticism throughout; to Dr. W. Holmes for the help in growing the plots and to other members of the Technical Chemistry Department.

C O N T E N T S.

INTRODUCTION.

The natural food of farm livestock, whether kept for milk, meat or work is pasture herbage. In northern climates many animals are wintered indoors and even the hardier types which remain outside require to be fed additional rations to supplement the scanty winter herbage. This involves building up, or buying, a store of forage for winter feed. In countries where spring and summer rainfall encourages good growth of grass the traditional method to meet the forage requirement has been to make hay. The disadvantage of this system is that the product has often a very low nutritional value and a major policy change in farming in the last two decades has been the general appreciation of the feeding value of young leafy grass.

As early as 1866, Wilson drew attention to the difference existing in the chemical composition of grass at various stages of its growth but these interesting investigations do not appear to have been followed at that time, and the recent rapid progress in grassland development can be ascribed chiefly to Woodman and his collaborators at Cambridge, Stapledon and Fagan at Aberystwyth and Watson and his co-workers at Jealotts Hill. These workers emphasised two aspects of herbage development, the need to know the chemical composition and nutritive value of different grasses at different stages in their life cycles and the modern methods of grassland management, including the use of artificial fertilisers, which would result

in improved yields of the best types of grasses. At the same time traditional grazing policies were examined and, in some cases, improved.

Along with the newer agricultural knowledge came improved methods of keeping the short leafy grass for winter feed. Machines capable of drying it, and more knowledge of the factors involved in making it into silage increased the demand for grass of the right quality. It was early found that although the composition of different types of grasses and forage legumes may vary slightly, the over-riding factor was the stage of growth of the plant. At first sight it might appear that maximum yield of herbage would result for allowing the grass to reach maturity but such a view would take no account of the ability of grasses, in particular, to respond to frequent cutting by the production of new growth. Moreover it has been shown that in mature grass the digestibility of the more fibrous material is lower than in younger grass, and this, together with the much lower protein value of mature grass, makes its nutritional value very poor. The carotene content of older grass decreases during growth in a similar manner to the protein and, whilst this constituent is not the criterion by which herbage should be judged, a high carotene content would certainly have a useful health-promoting effect. On the other hand, haymaking will always play a necessary and important

part on farms where livestock are kept, and hay should be looked upon as the natural complement of the young, high protein grass grown and conserved to replace concentrates and not, mistakenly, compared with it.

If some such system of grassland management was followed, probably using nitrogeneous fertilisers to increase both yield and quality of the sward; conserving young grass at the right stage of growth and making hay of the mature herbage, it would be of interest and importance to appreciate not only the obvious physical difference between grasses intended for different purposes but also their chemical composition at various stages. In the following pages an attempt has been made to carry out the latter objective. In the first section a consideration of the routine chemical methods which are common to any such analytical study has been made. This is followed by a report of the grassland investigation proper, planned in the following way.

A number of grasses of the kind used in this locality (S.W. Scotland) for hay production were sown on land in good heart but to which no fertiliser of any kind, either natural or artificial, was subsequently applied.

It was felt that although the results fromsuch herbage would be less than could be obtained by better management, yet at the same time they would be not too un-representative of the grass on many present

day farms. These grasses were allowed to grow unchecked by cutting or grazing and the weekly samples were never taken twice from the same area. In this manner the chemical composition of the grasses was followed from beginning to end of a growing season, the results providing the material reported in the second section. They provide a clear indication of the loss in feeding value which would result from missing the correct cutting stage either for hay or conservation and the importance of a leafy herbage is emphasised by analyses of the separated grasses.

Having established a type of baseline from which any changes brought about by different grassland management could be observed, attention was directed to the effect of applying an artificial, nitrogeneous fertiliser to two types of pasture. These were allowed to grow only to the physiological stage known to be economically worth-while for grass drying, about ten inches high, which comprises between very high protein grass too short to handle and longer grass of too low a nutritive value.

These two very different kinds of grassland policy, representing, as it were, minimum and maximum attention, give an indication of how careful planning could assist the farmer to meet his annual forage demands. Assuming, however, that a certain amount of the high protein grass would be conserved by drying, other problems arise which require investigation if

avoidable losses after the grass has been grown are to be minimized. These are dealt with in the final section and are concerned with the preparation of the grass for the drier, its treatment at fairly high temperatures and the changes in one constituent, carotene, which are likely to occur during storage. As in many other farming operations, the best possible results have often to be sacrificed because of other considerations, such as excessive cost, too high labour requirements or too great an expenditure of time and a good compromise is the best that can be obtained. Grass drying falls into this category of operation and the work here presented gives data from which such a compromise could be worked out.

SUMMARY OF RESULTS.

Part I. Analytical methods.

The moisture, crude protein and carotene contents of fresh grass were measured directly after cutting. In some cases, a representative part of these samples was dried and the true protein, ash, fibre and ether extractive later determined. Other dried grass samples were analysed for residual moisture, crude protein and carotene. Some of the methods normally used for estimating carotene, crude fibre and true protein were subjected to critical consideration and comparison.

Part II. The chemical composition of fresh grasses.

An established growth of timothy grass (*Phleum pratense*. L.) in its second year was cut at weekly intervals and the chemical changes determined. As the season advanced, protein, carotene and ether extractives decreased, rapidly at first, whilst fibre showed a regular increase over the same period.

A number of first year grasses of the type used for hay production were similarly examined and showed that considerable differences existed in their rate of establishment. Timothy developed most quickly, meadow fescue (*Festuca elatior*. L.) most slowly, with perennial ryegrass (*Lolium perenne*. L.) and cocksfoot (*Dactylis glomerata*. L.) equal and intermediate. Comparison of these four grasses and the second year timothy on a growth basis showed little difference in crude protein and carotene contents. A highly

significant correlation existed between crude protein and carotene in all fresh samples.

As a result of the analysis of leaf, stem and whole plant it was concluded that mechanical separation with a view to providing a leaf concentrate type of meal from older grass would not be practicable.

Part III. The effect of manurial treatment on grasses.

Nitrochalk was applied to a short ley and an established pasture in different amounts and at different times in the year. Of the nine treatments used, heavy spring dressings produced more material and higher protein and carotene contents in the short ley than the same or smaller quantities applied later in the year. Less variation was observed between the effect of the different manurial treatments in the older pasture although the general tendency was for it to respond best to heavy, spring dressings in a similar manner to the younger ley. The high correlation already noted between crude protein and carotene was again found.

Part IV. The carotene content of dried grass.

It has been shown that loss of water from cut grass by field wilting, whilst desirable from a drying point of view, results in considerable loss of carotene from the grass. A maximum wilting period of one to two hours, depending on weather conditions, is advocated if a high carotene grass meal is desired.

Normal commercial practice in producing and bagging grass was found to cause only minor loss of carotene but major losses occurred in storage due to air oxidation. These losses were increased by storage at high temperatures and could only be prevented entirely by removal of the atmospheric oxygen prior to storage. The beneficial effect of reducing the volume of oxygen available is discussed.

PART I. ANALYTICAL METHODS.

The forage material used in the succeeding sections has been grass, normally from first and second year leys, either in the fresh state soon after cutting or as a powder after drying and grinding. In almost all the analytical processes the physical state of the grass did not involve any change in method other than allowance for the smaller weight of dry matter in the fresh samples. The exception to this was in the estimation of carotene, where completely different techniques for fresh and dry grass were employed to obtain the carotene in solution prior to isolation and measurement.

The analysis of grass samples has involved the determinations of the following quantities:-

1. moisture.
2. total nitrogen.
3. protein nitrogen.
4. ether extractives.
5. crude fibre.
6. ash, calcium and phosphorus.
7. carotene.
8. length of the fresh grass material.
9. leaf to stem ratio.

Of these, only the methods for the determination of protein nitrogen, crude fibre and carotene require any discussion, normal practice being followed for

the others.

It should be stated at this point that all determinations other than moisture are reported on a moisture free basis to facilitate comparison between samples of varying moisture content. Where dried grass was used it was normally ground to pass 60's sieve but where it was used as test material to compare analytical methods it was ground to pass 80's sieve to lessen errors arising from lack of homogeneity.

1. Moisture.

Small samples of 1-2 g., ground or chopped to a fine state, were dried in dishes in an electric air oven for three hours at 102°C. When larger quantities, 60-100 g., were necessary, the samples were laid in metal cylinders of suitable size fitted with gauze bottoms in the same type of oven for not less than seven hours.

Although not primarily designed for the purpose of determining moisture contents, but on occasions used for this purpose, a vertical forced draught electric drying oven running at 110°C. was found to reduce 100-400 g. fresh material to a constant weight in 45 minutes. Percentage moisture content has been measured as weight of moisture lost from 100 parts of original material, (percentage dry matter where used is, of course, 100 minus the percentage moisture content).

2. Total nitrogen.

Sufficient sample to contain about 0.4 g. dry material was digested by the normal Kjeldahl technique (21) with copper and selenium as catalyst. Three hours digestion after removal of all carbonaceous matter was sufficient to give constant values for nitrogen. On the assumption that pure grass protein contains 16% nitrogen the factor of 6.25 was used to convert total nitrogen to crude protein.

3. Protein nitrogen.

The method for determining true protein nitrogen by formation of a copper protein compound as recommended by the Association of Official Agricultural Chemists (2) has been criticised in recent years as being insufficiently specific. In view of this it was decided to compare the A.O.A.C. procedure with a tannic acid precipitation method developed by van Roth (42).

Briefly, the details of the two methods are as follows.

(a) A.O.A.C. method.

A cuprous hydroxide suspension containing about 0.5 g. $Cu(OH)_2$ in 5-10 ml. is prepared by alkaline precipitation from copper sulphate solution in water containing 5 ml. glycerol per litre. 0.7 g. dried grass is boiled in 100 ml. water for 5 mins. and a volume of cuprous hydroxide suspension containing 0.5 g. $Cu(OH)_2$ is added while the liquid is thoroughly stirred. When cold, the precipitate is filtered

Table 1.

Estimation of Protein Nitrogen.

Sample	Total N (%)	A.O.A.C. - $Cu(OH)_2$		van Roth - tannic acid	
		Protein N (%)	N.P.N. (%)	Protein N (%)	N.P.N. (%)
Ryegrass	2.386	2.163 ± 0.189	0.223	2.087	0.299 ± 0.235
Cocksfoot	2.473	2.213 ± 0.189	0.260	2.086	0.387 ± 0.235
Fescue	2.271	2.122 ± 0.189	0.149	2.045	0.226 ± 0.235
Timothy	3.109	2.829 ± 0.189	0.280	2.618	0.491 ± 0.235

washed and transferred in the filter paper to a Kjeldahl digestion flask. 10 ml. of 40% sodium sulphide are added to precipitate all the copper and digestion carried out in the normal manner. Phosphate is not present in the grass in sufficient quantity to require decomposition with alum. The nitrogen found, multiplied by 6.25 gives the amount of true protein present.

(b) van Roth method.

50 ml. of a tannic acid solution containing 40 g. tannin plus 1 ml. concentrated sulphuric acid per litre, are heated for 30 min. on a boiling water bath and are then poured on to 0.4 g. dry grass meal. After 10 min. on the water bath the suspension is allowed to stand for 1 hour and is then centrifuged. The precipitate is washed with water, stirred and centrifuged successively for a further six times and finally transferred to a Kjeldahl digestion flask. The nitrogen present is determined in the usual manner and, after multiplication by the factor 6.25, reported as true protein. This method is more particularly suited to dry material and has been used in this way in the later sections of this work.

From the total nitrogen and protein nitrogen data a figure for non-protein, or amino nitrogen, is obtained by subtraction.

Table 1 gives the results of experiments comparing both methods, using four different species of young,

spring grass. Both methods placed the non-protein nitrogen contents in the same order but with very considerable differences between actual values. The figures obtained by the A.O.A.C. method were much lower than by the tannin method. During the estimation it was noticed that both methods suffered from appreciable quantities of grass particles floating on the surface of the liquors without apparently taking part in the extraction. This defect occurred to a greater extent in the A.O.A.C. method and since such particles, still containing non-protein nitrogen would be filtered along with the precipitate and subsequently estimated as protein nitrogen, this figure might therefore be expected to be erroneously high. It was found that adding 2 ml. 10% sodium ricinoleate to the grass before pouring on the tannic acid, in van Roth's method, wetted the grass and prevented particles from floating on the surface. Replication was worse in the A.O.A.C. method than in that of van Roth, where the maximum deviation from the mean was about the same as that in the determination of total nitrogen and may be ascribed largely to slight variations in the finely ground grass sample. Any non-protein nitrogen constituents determined as true protein in the A.O.A.C. method, as suggested by van Roth, would also have had the effect of increasing the value for this fraction with consequent diminution of the non-protein nitrogen.

On this evidence it was decided to use the method of van Roth, with the addition of preliminary wetting, in the estimations of true protein reported in Part 2 of this thesis.

4. Ether extractives.

The term "ether extractives" is preferred to "crude fat" since the substances removed from grass by boiling in ethyl ether will include organic acids, sterols and pigments in addition to true fats.

Dried material was used in all determinations of ether extractives because its smaller bulk made for easier handling. Moreover, a large amount of fresh material could be dried and ground to a small volume of powder which, in turn, allowed the preparation of a more uniform sample for analysis.

Using a Soxhlet apparatus with ground glass joints and previously de-fatted thimbles, the dried grass was continuously extracted for a minimum of nine hours with redistilled ethyl ether. At the end of this time the ether was completely distilled from the receiving flask under reduced pressure and the flask dried at 102°C. to a constant weight, removing ether fumes from the flask after the first hour and second hour with dry air. The increase in weight of the flask was calculated as ether extractives on the moisture-free weight of grass meal used.

5. Crude Fibre.

In this work crude fibre has been taken to mean

the plant material remaining after the removal of crude protein, ether extractives and soluble carbohydrates from the grass. It will consist of a mixture of celluloses, lignins, suberins and pectins in proportions varying with the age of the plant.

Until recently most published methods of fibre determinations involved successive acid and alkaline digestions, entailing considerable handling of small samples with all the attendant chances of experimental error. The method of Whitehouse, Zarrow and Shay (61), using a single extraction reagent, represents a distinct improvement where a large number of samples require analysis, providing the results are at least as accurate as standard methods. To investigate this a sample of pure, second year, timothy hay grass, taken in July, was dried and ground to a fine meal.

(a) A.O.A.C. method.

The method given in the 5th Edition of the A.O.A.C. (5) was taken as representing the acid-alkaline type of extraction and was carried out as follows.

1-2 g. dried grass was freed of all fatty material by the Soxhlet extraction method given above and dried for 5 hours at 102°C. 1 g. was weighed into a 750 ml. flask and boiled for 30 min. with 200 ml. 1.25% sulphuric acid (w/v). The suspension was filtered through asbestos which had been previously

Table 2.

Estimation of Crude Fibre.

No.	% crude fibre	
	A.O.A.C.	Whitehouse, Zarrow and Shay
1	38.22	38.50
2	38.10	38.31
3	38.10	38.14
4	38.23	37.76
5	38.81	38.52
6	38.19	38.10
7	38.34	38.75
8	38.35	38.77
Mean	38.29	38.38
S.E	38.29 ± 0.08	38.38 ± 0.09

digested for 8 hours with 5% sodium hydroxide followed by 2N hydrochloric acid, washed, dried and ignited. The grass and asbestos were washed back into the flask and boiled for a further 30 min. with 200 ml. of 1.25% sodium hydroxide (w/v). At the end of this time the contents of the flask were filtered through a Gooch crucible, washed, dried for 5 hours at 103°C. and weighed. The crucible was then heated in a muffle at about 550°C. to a constant weight. The difference between the dry and ignited weights was calculated as crude fibre on the moisture-free grass meal.

(b) Method of Whitehouse, Zarrow and Shay.

As before, about 1 g. fat-free grass meal was extracted, this time with 100 ml. of a solution containing 500 ml. glacial acetic acid, 50 ml. nitric acid (S.G. 1.42), 20 g. trichloroacetic acid and 450 ml. water. The suspension was boiled under reflux for 40 min., care being taken to prevent bumping in the early stages. It was then cooled and filtered through a Gooch crucible prepared with asbestos previously treated as in (5a) above and washed with water. The crucible was dried, heated in the muffle and crude fibre reported as before.

The results of octuplicate determinations on the same sample by the two methods are shown in Table 2. There was obviously little difference between them, and since the manipulative time required by the method of Whitehouse et al. was only about half that required

by the A.O.A.C. method, the former was used in the present work.

6. Ash.

5 g. dried grass meal was ignited in a wide silica basin over a bunsen burner until thoroughly charred. The basin was then transferred to a muffle furnace thermostatically controlled at 580-600°C. At the end of an hour the basin was removed, cooled and the ash moistened with a few drops of water and nitric acid. After drying on a sandbath the basin was again heated in the muffle for a further hour. At the end of this time all carbon had disappeared and no further loss in weight occurred with more prolonged heating.

7. Calcium and phosphorus.

The ash obtained as in para.6 or from dried grass moistened with 5 ml. 20% magnesium chloride and then treated as in para. 6, was dissolved in warm 50% hydrochloric acid and transferred to a 100 ml. volumetric flask. After neutralisation with strong ammonia (to methyl red) the contents of the flask were again made slightly acid and diluted to volume.

Calcium was determined on duplicate 30 ml. aliquots of this ash solution by the usual method of oxalic acid, oxalate and sodium acetate additions. The oxalate precipitate was allowed to stand overnight and was then washed four times with 3% ammonia, being centrifuged after each washing. After decomposition of the oxalate with sulphuric acid the calcium was estimated by titration with 0.05 N potassium

permanganate at 50°C. and reported as a percentage of the moisture-free dry grass.

Phosphorus was determined by the method of Fiske and Subba Row (17) on the same ash solution. As the phosphorus was determined colorimetrically using a "Spekker" absorptiometer, a calibration curve compiled from solutions containing known amounts of phosphorus was first prepared.

A solution containing 60 mg. phosphorus per litre was prepared from potassium dihydrogen phosphate and the required volume pipetted into a 100 ml. flask together with 50-60 ml. water. A molybdate solution containing 25 g. ammonium molybdate and 500 ml. 10 N sulphuric acid per litre was prepared, and 10 ml. added to the 100 ml. flask followed by 4 ml. of a freshly prepared 1.2.4. amino-napthol-sulphonic acid solution containing 2 g. 1.2.4. aminonaptholsulphonic acid, 120 g. sodium bisulphite and 24 g. sodium sulphite per litre. After dilution to 100 ml. the reagents were allowed to stand in the laboratory for 10 min. and the blue colour then measured in the Spekker absorptiometer using a red filter with maximum absorption at mμ. Six points judged to be adequate to cover the range of phosphorus likely to occur in the grass samples were obtained and a smooth curve was drawn through them and the origin. It was checked that a blank determination using all the reagents except the phosphorus solution gave a negligible Spekker reading.

Usually 5 ml. of the ash solution, neutralised to phenolphthalein with sodium hydroxide and treated with the same additions as the standard solutions to produce the blue colour were adequate to give a reasonable reading on the Spekker. From the calibration curve the phosphorus in the ash solution and hence in the original grass powder could be calculated.

Carotene.

Carotene in plant materials forms part of the cell chromoplasts and any method for the estimation of this pigment must first provide a means of extracting it. When considering fresh plant material all the multitudinous methods to be found in the literature fall into one of two groups, the first of which utilises chemical degradation of the tissue, usually with the application of heat, and was popular with earlier workers whereas the second favours some form of mechanical disruption of the material. In both systems the final aim is to arrive at a solution of the separated carotene, usually in light petroleum. Advocates of each method claim advantages for their own way of working and point to disadvantages in the other type of extraction. Very briefly, the proponents of chemical degradation aver that by rapidly submitting the plant material to the attack of hot reagents any possibility of atmospheric oxidation is minimised whereas mechanical grinding to break down tissue is likely to provide just such opportunities.

This argument is met by the opposite view that the necessary grinding is done under the surface of organic solvents which contain a minimum of dissolved air and in which, in fact, a suitable antioxidant such as hydroquinone can be incorporated. Moreover, by avoiding even relatively short periods of heating with concentrated alcoholic alkali the chance of forming coloured resinous compounds separable only with difficulty from the plant pigments is avoided. This latter criticism was, perhaps, more justified before chromatographic separation of carotene from chlorophyll and xanthophyll replaced the classic methods of differential solubilities in two-phase systems employed originally by Wilstatter & Stoll (62).

Booth (6) has critically reviewed the whole problem of extraction and estimation of carotene and has drawn attention to the chief sources of error up to the colorimetric stage. These he considers to be:-

1. losses of carotene before extraction begins,
2. destruction during and after extraction,
3. incomplete extraction,
4. manipulative losses,
5. incomplete removal of other pigments and
6. chromogenesis and isomerisation.

The first four of these would lead to low results whilst the last two would give high results. Losses of carotene before extraction can be minimised by starting the analysis immediately after cutting, avoiding

tissue damage until the extraction proper begins and working in subdued light. During extraction, exposure to sunlight can lead to considerable losses by photochemical destruction which, according to Pepkowitz (40) is catalysed by chlorophyll. From these two sources alone, in extreme cases, as much as 50% carotene may be lost. The difficulty with which carotene is extracted by some solvents is, according to this author, not sufficiently recognised and contributes to incorrect results. Experimental losses are more likely to reach high proportions in processes involving a large number of laborious manipulations such as occur in the phasic separation of carotene from the other pigments by solvents. The modern methods employing chromatography minimise errors from this source. Similarily, abandonment of separation by solvents has removed the erroneously high results given when incomplete separation of, for example, non-hydrolysed xanthophyll esters occurred. Some isomerisation of β-carotene seems unavoidable, but the formation of pseudo carotenoids during treatment with alcoholic KOH can be avoided by employing a method using little or no heating.

The experiments described in Part 5 of this work were the concluding part of a collaborative investigation on the effect of manurial treatment on grassland, in which the previous carotene estimations had been carried out by one of the methods employing chemical saponification, devised by Seshan & Sen (44). In

order to keep the carotene data on a directly comparable basis, so far as analysis was concerned, this method, as modified by Austin & Shipton (4) was followed using phasic separation although its disadvantages were clearly recognised.

An early stage of Seshan & Sen's method is to reflux the fresh grass with alcoholic potassium hydroxide for half an hour, and Austin & Shipton have shown that there is less chance of resin formation if the grass is refluxed first with aqueous alkali and then triturated on a sintered filter with the alcohol. Preliminary trials with a number of grass samples showed that there was, in fact, little difference between carotene values obtained by the two methods for the same sample. In view of the sound underlying principle of the modification, the Austin & Shipton method was used to determine the carotene values reported in Part 4.

Before continuing with the estimation of carotene in fresh grass, a comparison was undertaken between this method and that developed by Booth to avoid the many sources of error which he had listed.

Method of Austin and Shipton.

The sample of grass was finely chopped in a darkened room and about 2 g. quickly weighed into a 250 ml. conical flask. 10-15 ml. 20% potassium hydroxide was added and the contents of the flask brought quickly to the boil and kept boiling for 2-3

min. under a reflux condenser. The flask was cooled, six volumes of absolute ethyl alcohol added and the liquor refluxed for 30 min. out of direct daylight. At the end of this time the grass residues were filtered on a Jena 3G1 sintered glass filter and stirred with successive volumes of alcohol until the filtrate was colourless. The filtrate was transferred to a separating funnel and the alcohol content adjusted to 65%. Light petroleum (B.P. 40-60°C.) was then used in a similar manner on the filter until no further colour was extracted into the filtrate. The petroleum extract was then added to the alcohol in the separating funnel and shaken. After separating the petroleum layer the alcohol fraction was shaken twice with fresh volumes of petroleum which after separation were added to the first petroleum fraction. The bulked petroleum extracts containing carotene and xanthophylls were washed three times with an equal volume of water to remove alkali, and were then shaken with 90% methyl alcohol to remove xanthophylls. The petroleum solution was then dried over anhydrous sodium sulphate, made up to a standard volume and the depth of colour determined on the Spekker absorptiometer. A calibration curve had previously been prepared by measuring the colour of a number of petrol ether solutions containing known amounts of pure β-carotene. The latter dissolved in arachis oil had been obtained from the Medical Research Council.

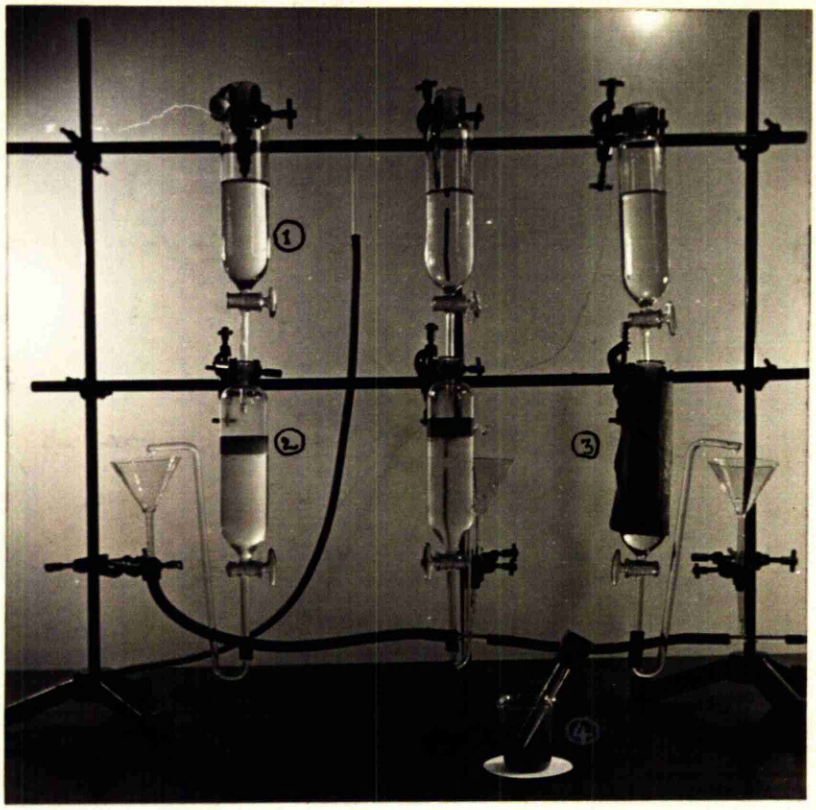


Fig 1.

Removal of acetone from pigment solution.

1. Washing water.
2. Solution of pigments in acetone-petrol ether.
3. Light shield.
4. Extraction of pigments by grinding.

Booth's method.

About 0.5 - 1.5g. freshly cut grass, chopped to about 3 mm. lengths was quickly weighed into a small glass mortar in a darkened room. An equal weight of quartz sand was added, 5-8 ml. of a solution of light petroleum (B.P. 40-60°C.) and acetone in the ratio of three volumes to two, in which 1 g. hydroquinone had been dissolved per litre, was run in and the grass ground for 1-2 min. The solvent was decanted into a shaded separating funnel half filled with water. The funnel was fitted with an S tube at its lower end, as shown in Fig. 1. to maintain a constant level. Two or three more successive grindings with solvent released all the pigments from the grass. To remove the acetone, the bulked solvent extracts were then washed for approximately one hour by water dropping through the solvent layer. At the end of this time the funnel was rolled to wash down any solvent which had splashed on to the upper walls and the water was then run off. The petroleum layer at this stage contained all the grass pigments in solution. These were next separated by adsorption on a column of Hopkin & William's brand "Alumina, activated for chromatographic analysis". This adsorbent has been used throughout, and it has been found that a further heating at 102°C. prior to use considerably aids pigment separation. In this way all the chlorophyll and xanthophyll can be retained in a

narrow band of 3-4 mm. at the top of a 4 cm. column even after elution of the carotene with light petroleum containing 3% (v/v) of acetone. As many as six separate determinations can be made on one column, provided that all traces of acetone in the pigment solution have been thoroughly removed in the washing process and that the column is cleared of any acetone after each previous elution by washing with 20-30 ml. light petroleum. The eluted carotene was made up to 50 ml. and estimated by means of the Spekker absorptiometer and the calibration curve. At no time in the whole process did direct daylight have access either to the plant tissue or the solution of pigments.

The limiting factor in producing good replicates by either of these two methods is in sampling the original fresh material. A large bulk of grass cannot be chopped and adequately sampled at leisure without involving carotene losses that would considerably outweigh any gain in accuracy of replication; consequently all the inaccuracies attendant on the use of small samples are reflected in the scatter usually obtained in replicated carotene values of fresh grasses. As will be seen later (Part 5), the effect of taking say, a slightly greater proportion of stem in one sample than another materially affects the resulting carotene value. In all determinations duplicate samples were analysed

Table 3.

Estimation of Carotene.

Sample	Carotene (mg./kg. dry wt.)			
	Austin & Shipton		Booth	
		Mean		Mean
1	46 48	47	43 46	45
2	53 55	54	48 53	51
3	71	-	81	-
4	104 114	109	103 107	105
5	125 129	127	124 132	128
6	176 182	179	150 164	157
7	225 233	229	195 205	200
8	270 278	266	246 256	251
9	360 367	364	327 329	328

and whilst results were usually within ± 5% occasional results were more divergent. In such cases wherever possible, i.e. where no greater change in carotene content owing to the lapse of time would be expected, further analyses were carried out, but this was not always practicable.

In Table 5 the carotene values obtained by Austin & Shipton's and Booth's methods are given for several fresh grasses. In this table and in all sections of this work, carotene is expressed as mg./kg. of dry material in order to facilitate comparison between samples of different moisture content.

It will be seen that although at low concentrations the difference in results obtained by the two procedures was not great, Austin & Shipton's method gave greater values at the higher concentrations of carotene. Assuming that manipulative losses were similar in both cases, it would appear that with grass containing large amounts of carotene, and therefore probably other pigments, phasic separation did not completely eliminate some of the other pigments. In addition to the liklihood of more accurate values by Booth's method, the considerably less manipulative work, the greater precautions against chance losses and its easy adaptation to multiple determinations all pointed to this method as being the best which was investigated, and in all subsequent work involving the determination of carotene in fresh grass, this was

the method used.

Unfortunately Booth's method is not particularly well adapted for the determination of carotene in dried grass, although by suitable moistening the powder the pigments can be extracted after considerable grinding. There have been, however, many methods proposed for this purpose. As with fresh grass, the first problem is to get the carotene into a solution whose strength can be measured. Most published methods involve extraction of the dried grass with some solvent or mixture of solvents, with or without heating, although some employ much the same conditions of alkali digestion as with fresh grass (16), (19), (41). A Committee convened by the British Grass Drier's Association (8) compared three methods which used ethyl ether, light petroleum and alcoholic potassium hydroxide respectively and concluded that extraction with light petroleum (B.P. 40-60^{0}C.) for one hour gave the best results. Wall & Kelly (52), used a mixture of light petroleum and acetone, similar to that employed by Booth for fresh grass, in which the dried grass was refluxed for half an hour. These two methods were in the process of being compared when Nelson (39) published results of further work by re-constituted Committee of the Grass Drier's Association.

A comparison of the 1941 method of the Grass Drier's Association with that of Wall & Kelly showed that much lower values were obtained by the latter.

This was apparently because extraction of the dried grass for half an hour was insufficient to liberate all the carotene. Tests showed that, even after two and a half hours the carotene of grass meals was not completely extracted. Apart from the length of time taken to extract all the carotene the increased risk of isomeration of β-carotene made Wall & Kelly's method unsuitable.

In his report, Nelson drew attention to defects in the 1941 method of the Grass Driers' Association. This was capable of including 50% of biologically inactive carotenoid substances in the determined value of β-carotene and also of incompletely removing all the chlorophyll from the final carotene solution. In view of these failings the committee had carried out further work and finally recommended a new method for the estimation of carotene in dried grass. This method employed light petroleum (B.P. 80-100°C.) for pigment extraction followed by adsorption on de-fatted bone meal. Carotene was then eluted with light petroleum (B.P. 40-60°C.) and estimated colorimetrically. Although on investigation the method was found to be generally satisfactory, it appeared that a more easily reproduced adsorbent than bone meal was desirable and for this purpose activated alumina of the type already mentioned was used. This necessitated elution with a slightly more polar solvent than light petroleum alone and a mixture containing 3.5% acetone in light

petroleum (B.P. 80-100°C.) was employed.

The method ultimately used for the determination of carotene in dried grass was therefore as follows:

> 0.5 - 1 g. dried grass meal was weighed directly into a 250 ml. Kjeldahl flask. 60 ml. light petroleum (B.P. 80-100°C.) were added and the flask heated for one hour in a briskly boiling water bath. The long neck of the Kjeldahl flask acted as an air condenser. The flask was then cooled and the liquid decanted on to a 4 x 1 cm. column of activated alumina. The flask and residues were twice rinsed with small volumes of light petroleum (B.P. 80-100°C.) which were added to the column. Suction was applied to the alumina column when the first liquid was decanted and, without letting the column become dry at any time, all the pigment solution was drawn through. After changing the receiver the carotene was eluted with 3-5% acetone in light petroleum. The carotene solution was measured on the Spekker absorptiometer and the amount of carotene calculated from the calibration curve.

6. Grass lengths.

In the work described in Part 3 a good estimate of the state of growth at different times of the year was required. For this purpose one hundred plants in a well mixed sample, taken from the bulk of material cut for other analyses, were measured to the nearest inch. The results were collected into groups, each group covering a four inch range and the number of plants falling within each group expressed as a percentage, as shown in Table 4.

(a) Leaf-stem ratio.

The leaves of the hundred plants used in the length measurements were cut where they joined the main stem and weighed. The stems were also weighed and, after correction for any differences in moisture

content, the ratio of weight of leaf to unit weight of stem calculated. In view of the considerable morphological differences developing during the course of the investigation it was decided to make no attempt to differentiate between leaf sheath and stem proper. The carotene content of leaf sheath is always less than that of true leaf but the proportion of sheath to leaf by weight is small and the error involved was not great.

PART 2.

THE CHEMICAL COMPOSITION OF FRESH GRASSES.

In the Introduction it was mentioned that the work in this section was undertaken mainly to provide data complementary to that obtained from grass plots cut at regular intervals during the manurial trials. It was felt that a better appreciation of the value of fertiliser and of grassland management would be forthcoming if comparable analyses were carried out on grass which had received no fertiliser and had been allowed to grow without check by grazing or cutting.

There was also a secondary consideration. For some time past the difficulty of drying all the grass when it reached the most nutritious, leafy stage during the early summer flush period had been very apparent. At such a time as this the grass grower has either to leave some grass and later dry it at a considerably more mature stage than desirable or to utilise it for silage or hay. From such excellent work as that of the Aberystwyth school under Fagan (13) it was obvious that the nutritional value of the leaf and stem of grasses would vary considerably over the normal growing period. Bearing in mind these two factors, it appeared desirable to consider the possibility of separating the leaves and stems of grasses which had grown past the stage at which they would normally be cut for grass drying. In this way

a high protein, high carotene, low fibre meal could still be obtained from relatively mature grass. The obvious difficulties involved in producing any form of mechanical separator were sufficient to make the provision of data to showwhether such a separation would be economically useful, one of primary importance. Hence the studies on non-fertilised, free-growing grasses were extended to include examination of the chemical composition and amount of both leaf and stem as well as of the plant as a whole.

Grasses used.

A strip of pure Scotch timothy sown the previous year was used for most of this work. At the same time four types of haying grass, Ayrshire perennial ryegrass, Danish cocksfoot, early fescue and Scotch timothy were sown in the spring of the year to afford a comparison with the second-year grass. The land on which all the experimental plots stood was well drained and in good heart, of pH 5.8 with no deficiency of minor elements. Hand-weeding was maintained on the first-year plots until the grass had provided good cover.

Cutting.

All the material examined was cut for the first time in the 1947 season and analysed on the same day, as uniform a sample as possible being taken at each cut.

The second-year timothy was cut once each week from May to the end of July and thereafter at three-weekly intervals until the end of September. The

first-year grasses were cut when they had reached a height of about 4 inches and thereafter at about fortnightly intervals.

Sampling and analyses.

The grass was cut at 9 a.m. at about one inch from the ground. It was covered with brown paper and taken to the previously shaded laboratory. 60 - 100 g. were dried at once to give a value for the bulk moisture as already detailed (p.10). From the bulk sample a smaller number of representative plants was taken and cut as quickly as possible into 2-3 mm. lengths. Duplicate samples of 2 g. of this material were weighed into thick walled glass beakers and used immediately for carotene estimation. Further 2 g. samples were then weighed into ground-glass, stoppered weighing bottles for later moisture and crude protein determinations. At the same time as these weighings were being made, the leaves and stems of another representative sample were separated and weighed. They were then similarly chopped and used for the estimation of carotene, moisture and crude protein. After the initial cutting in the field, no plant tissue was damaged in any way more than five to ten minutes before the start of the corresponding carotene extraction, and the last carotene determination of the day was commenced within an hour of the grass being cut in the field. Under these conditions it was found that the loss of carotene at the end of one

hour was negligible and in this way carotene losses prior to extraction were reduced to a minimum. This procedure was carried out with all samples throughout the year.

As soon as the carotene, moisture and protein determinations were started the lengths of a representative grass sample were measured, followed by the determination of the relative weights of leaf and stem.

During the summer months there was insufficient time to carry out estimations of ash, fibre, minerals etc., on the fresh grass and it was necessary to separate and dry sufficient leaf and stem at each cutting to provide 10-20 g. of dry material for analysis at a later date. This drying was done in a small vertical forced draught oven running at 110°C. The dried material was ground to a powder through an 80's sieve in a Christy & Norris laboratory model hammer mill and securely bottled.

Weather.

The West of Scotland usually provides excellent climatic conditions for the production of grass and, after a severe winter which retarded early growth, the season under consideration was no exception. Records obtained from the neighbouring meteorological station at Auchincruive showed that sufficient rainfall throughout May and June coupled with fairly high average temperatures provided weather suitable for normal growth of grass.

[redacted]

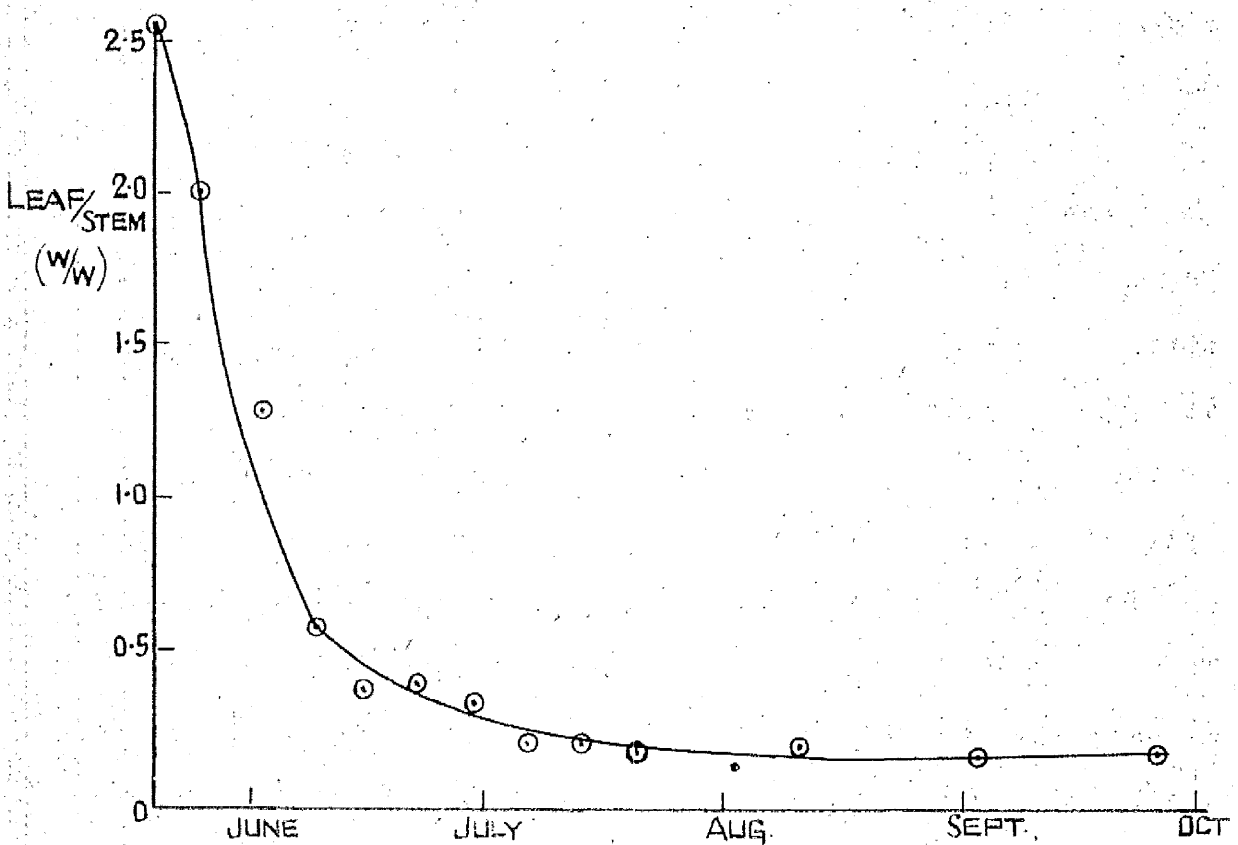
Table 4.

Growth of second year timothy.

Leaf to Stem Ratio (by dry weight)	Date 1947	Length of Plant (inches)									
		Below 6	6 to 9	10 to 13	14 to 17	18 to 21	22 to 25	26 to 29	30 to 33	34 to 37	38 to 41
		Percentage plants in each group									
2.57	May 20	32	59	9							
2.15	" 26	15	52	28	5						
1.30	June 2		16	27	23	23	11				
0.58	" 10			23	41	30	6				
0.29	" 16			10	29	47	14				
0.41	" 23				12	46	26	11	5		
0.55	" 30				17	21	21	23	18		
0.21	July 7					9	26	43	18	4	
0.20	" 14				4	15	27	37	15	2	
0.16	" 21						19	37	33	11	
0.19	Aug. 11					2	11	30	37	18	2
0.16	Sep. 3					4	21	29	34	8	4
0.18	" 25					14	22	14	27	20	3

FIG. 2.

LEAF-STEM RATIO IN 2ND YEAR TIMOTHY



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RESULTS.

The results of the analyses of the second-year timothy will be presented first. Table 4 shows the changes in height of the grass from May to September, 1947; the figures in each size column give the percentage of plants in a representative sample falling within this range of lengths. The ratio of leaf to stem (by weight) is included to amplify the growth data.

These figures show that from the early part of the year until about the end of June growth was regular and continuous, thereafter there was little change in height with the exception of small percentages at both extremes of length. Flower heads were visible in the stem sheath on June 2nd and were out by June 10th. After this date the heads were analysed separately and were not included in the leaf/stem ratio calculation. Seed shedding began about the end of August and was almost complete by September 3rd. Fig. 2 shows graphically the rate of change of the leaf/stem ratio over this period. The weights of both leaf and stem have been reduced to a moisture free basis for comparison. After a slow initial change the ratio dropped sharply during the period of maximum growth, when the stem was rapidly elongating, and quite quickly assumed an almost constant value. This type of curve will be seen subsequently to have close parallels in the rate of change of some of the plant

Table 5.

Chemical composition of whole second year timothy plants.

Date 1947	Moisture (%)	Crude Protein (%)	Carotene (mg./kg. dry wt.)	Crude Fibre calculated* (%)	Ash calculated* (%)
May 20	77.2	18.4	274	19.2	8.00
" 26	81.6	18.8	273	22.6	8.50
June 2	77.0	14.2	156	26.4	7.15
" 10	82.6	10.6	128	28.2	7.72
" 16	76.1	9.2	148	31.2	6.86
" 23	76.2	7.1	74	32.6	6.50
" 30	70.5	6.3	72	30.2	5.98
July 7	69.2	6.8	66	31.2	6.12
" 14	65.0	5.5	88	31.9	5.62
" 21	68.2	4.2	44	32.5	5.32
Aug.11	56.8	3.9	42	35.8	5.19
Sept.3	44.8	2.5	33	34.9	4.41
" 25	51.3	1.9	9	39.6	6.31

* Calculated from analyses of Leaf and Stem and Leaf/stem ratio.

Correlation coef. between protein and carotene. 0.974 (H.S).

Table 6.

Chemical composition of the leaf of second year timothy.

Date 1947	Moisture (%)	Crude Protein (%)	True Protein (%)	Carotene (mg./kg. dry wt.)	Ether Extracts (%)	Crude Fibre (%)	Ash (%)
May 20	76.6	21.7	18.6	343	3.79	19.1	7.09
26	78.1	21.5	17.6	480	3.91	22.0	8.06
June 2	78.4	17.2	16.0	341	4.73	23.8	6.51
10	77.3	15.2	13.3	251	4.45	24.9	9.55
16	78.0	19.5	16.9	369	4.14	26.1	8.01
23	72.7	13.7	11.4	259	3.55	27.1	8.02
30	60.5	12.3	9.2	170	3.26	26.9	8.76
July 7	66.9	11.6	9.3	208	3.08	26.9	9.05
14	56.5	11.1	9.4	203	3.24	30.6	8.96
21	72.0	10.7	9.4	120	2.93	30.2	8.90
Aug. 11	53.2	8.7	6.6	64	2.69	31.8	9.31
Sept. 3	20.8	6.1	5.1	33	1.24	33.8	9.03
25	23.8	5.9	5.0	14	2.05	35.9	9.59

Correlation coef. between carotene and protein, 0.894 (H.S.)

constituents over the same period. These ratios record only weights of leaf and give no indication of the physical and morphological changes proceeding concurrently. As early as 2nd June the primary leaves were browning at the tips and by 14th July both primary and secondary leaves were entirely brown and shrivelled. On 3rd September nearly all the leaves were brown and three weeks later the whole plants appeared brown and dry. In Tables 5 to 8 the accompanying changes in chemical composition are shown. Table 5 deals with the grass taken as a whole, excluding only the roots, Tables 6, 7 and 8 deal with leaf, stem and head respectively.

It can be seen from Table 5 that the plants started to increase in dry matter from the end of June onwards, reaching a fairly steady, comparatively dried-up state by September. The fall in protein and carotene was progressive from the end of May, although with both the decrease was most rapid during the period of maximum growth. The close correlation between protein and carotene content will be examined in more detail later in this section (p.47).

In Table 6 similar data for the separated leaves show, particularly in the values for protein, the same trend of progressive losses from May to September. The carotene contents are rather more irregular, although again gradual decrease from the high values of early summer is very apparent. The value obtained on 16th

Table 7.

Chemical composition of the stem of second year timothy.

Date 1947	Moisture (%)	Crude Protein (%)	True Protein (%)	Carotene (mg./kg. dry wt.)	Ether extracts (%)	Crude Fibre (%)	Ash (%)
May 20	83.8	14.1	11.1	86	2.87	23.5	9.95
26	83.1	14.1	11.5	112	2.77	23.9	9.46
June 2	84.2	11.4	8.6	41	2.46	29.7	7.95
10	81.6	7.4	6.1	45	2.03	30.7	7.37
16	72.9	7.6	5.9	71	2.56	32.6	6.65
23	71.4	3.8	3.5	32	1.62	34.9	5.91
30	63.1	4.4	4.2	32	1.72	31.7	5.01
July 7	63.7	3.4	3.3	35	1.52	31.9	5.59
14	60.4	3.4	2.7	58	1.32	32.4	4.96
21	60.5	1.9	2.0	13	1.18	32.7	4.76
Aug.11	56.1	2.2	1.8	34	0.64	35.7	4.35
Sept.3	49.5	2.3	1.7	14	0.78	34.9	3.66
25	50.5	1.6	1.8	12	0.49	40.2	5.71

Correlation coef. between carotene and protein. 0.943 (H.S.)

June is suspiciously high, but it will be noted that it corresponds to an equally high protein content. This suggests that the sample taken was not truly representative rather than that the estimations were faulty. As the plants became more fibrous the ether extractives decreased while the fibrous material increased. It may appear surprising that young leaves should contain such large amounts of fibrous material but the values recorded agree well with the figures given by Hosterman & Hall (27) who found, for the leaf of timothy grass, at the "headed" stage (corresponding to the stage existing on about 16th June) 24.5% fibre and at the "mature head" stage (c. July 7th) 25.9% fibre. The ash figures all appear rather low; no explanation can be offered for this unless lack of fertiliser application over two years could be responsible. Even lower figures still have been reported by Harold Hvidsten (28) whose values range from 6.5 in early June to 5.8 within two months.

It will be seen from Table 7 that all the constituents followed much the same course as the corresponding ones in the leaf analyses but, with the exception of fibre, at a lower level. After the end of July, moisture content, protein and carotene changed only slightly although fibre showed a slight rise. The comparable fibre figures for timothy stem from Hosterman & Hall (27) are "headed" (cf. June 16) 33.8% and "mature head" (cf. July 7) 41.6% crude fibre.

Table 8.

Chemical composition of the flower heads of second year timothy.

Date 1947	Moisture (%)	Crude Protein (%)	Carotene (mg./kg. dry wt.)
June 16	78.2	20.0	83
27	71.4	13.0	96
30	60.5	12.3	75
July 7	65.2	13.1	70
14	65.6	12.6	61
21	68.5	9.7	59
Aug. 11	45.7	12.0	44
Sept. 3	17.8	7.9	5
Correlation coef. between carotene and protein.		0.772 (H.S.)	

FIG 3

THE EFFECT OF SEASON ON THE PROTEIN CONTENT OF 2ND YEAR TIMOTHY

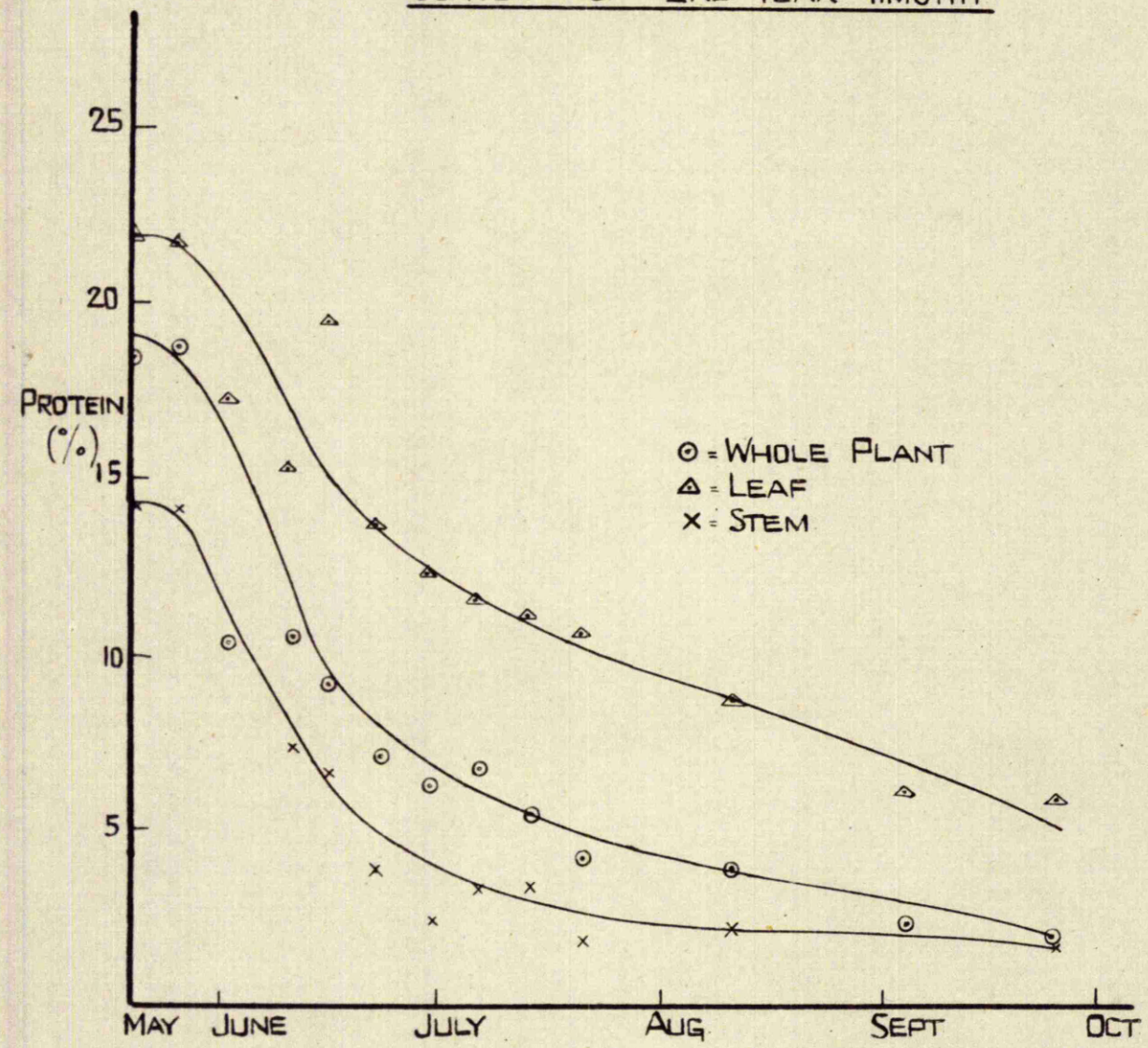

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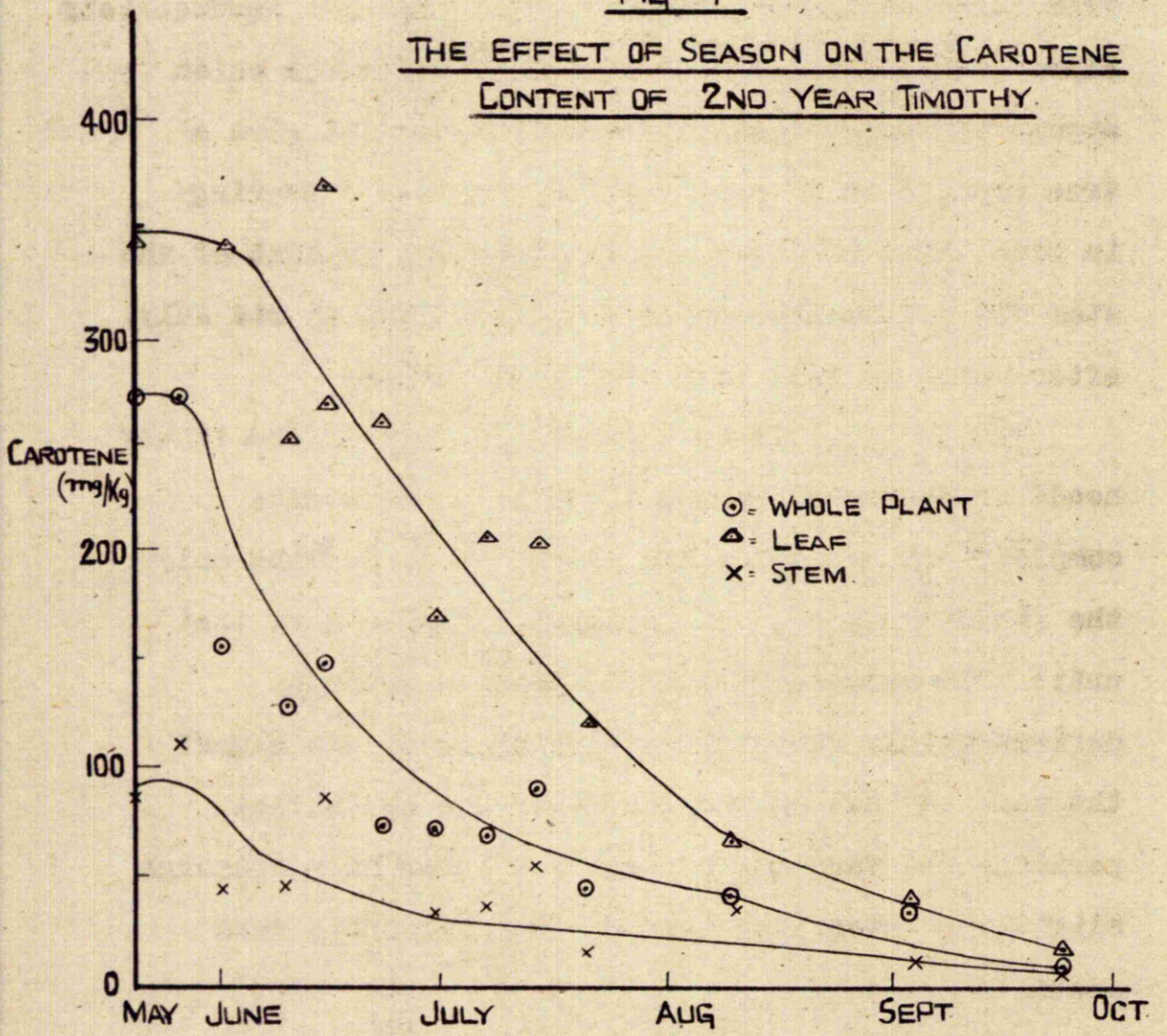
FIG. 4.

THE EFFECT OF SEASON ON THE CAROTENE CONTENT OF 2ND YEAR TIMOTHY.



The negative migration of mineral elements from plant to soil in the autumn mentioned by Deleanu (9) may account for the decrease in ash content at the end of the season.

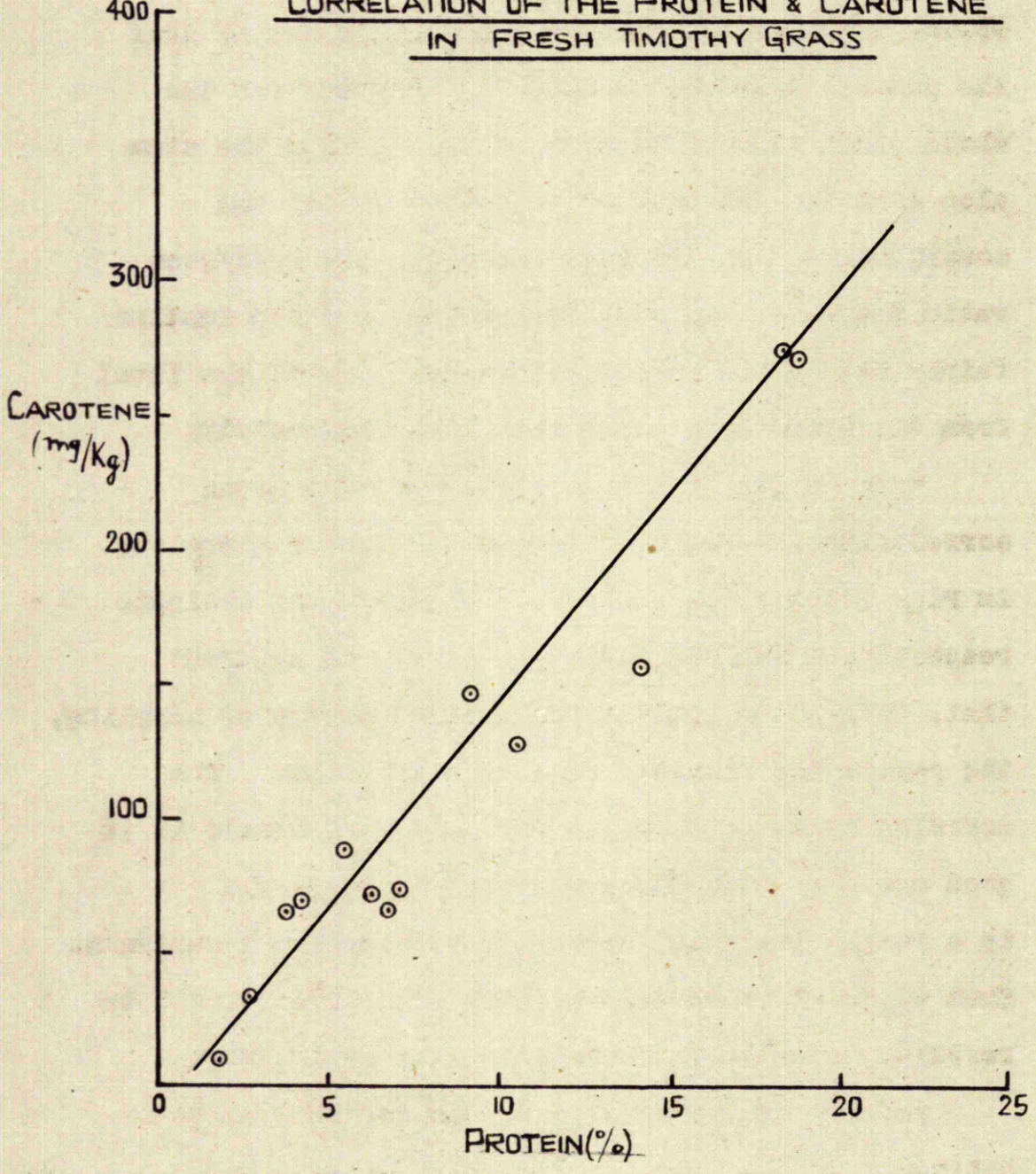
Extraction of such small amounts of carotene from somewhat fibrous tissue is not easy and inadequately representative sampling can result in figures which appear to indicate differences that may not give a true impression of the grass as a whole. Bearing in mind these limitations, the carotene content of the stem did not change appreciably from June to mid July, after which it fell to a negligible value.

The sudden fall in moisture content of the flower heads in September, shown in Table 8 represents complete dehiscence of the mature seed, leaving only the fibrous glumes. It is interesting to note that until this occurred the crude protein content, derived mainly from the developing seed, was almost the same as that of the leaf over the equivalent period. The carotene content was never high although slightly greater than that of the stem until seed shedding.

In Figs. 3 and 4 a comparison is made of the changes in crude protein and carotene occurring in the whole plant, the leaf and the stem. The data for the flower heads is not included since they would never be considered economically as a source of either constituent.

FIG 5.

CORRELATION OF THE PROTEIN & CAROTENE IN FRESH TIMOTHY GRASS



It will be seen that the general shape of all six curves is similar and follows the basic pattern illustrated in Fig. 2 of the changes occurring in the leaf/stem ratio. This is as might be expected. In spring when the plants have a preponderance of leaf the general level of protein and carotene for the whole plant is high because at such a time the stem also contains its highest concentration of the constituents. As the year advances, the leaf/stem ratio decreases and even though leaf protein remains fairly high until August, it cannot prevent the level from the plant as a whole from dropping rapidly.

Mention has been made already of the close correlation between protein and carotene content and in Fig. 5 these are plotted as abscissa and ordinate respectively for the whole plant. It is apparent that, within the limitations imposed mainly by sampling, the points lie sensibly on a straight line. The correlation coefficient is 0.974. This result is in good agreement with those reported by MacDonald (33) in a recent review of factors affecting grass analysis. This correlation between protein and carotene will be referred to again in connection with dried grass.

Turning to the first year grasses it will be noticed that the intervals between analyses were considerably greater than in the second year timothy experiments; this was, unfortunately, unavoidable. With the exception perhaps of the fescue grass,

Table 9.

First year Scotch timothy (Hay type).

Date	Average Height inches	Bulk Moisture (%)	Crude Protein (%)				Carotene (mg./kg. dry wt.)				Ratio
			Whole	Leaf	Stem	Head	Whole	Leaf	Stem	Head	Leaf/stem (dry wt.)
June 12	3.5	80.9	22.6	-	-	-	408	-	-	-	all leaf
20	4.5	83.4	22.0	23.6	13.8	-	405	484	91	-	mainly leaf
26	7	80.9	18.4	21.2	16.4	-	259	379	100	-	3.22
July 4	8	81.8	20.3	21.8	15.6	-	312	484	110	-	2.40
11	14	82.8	14.9	18.6	8.1	15.9	323	388	115	127	1.11
17		75.0	11.5	16.4	5.5	13.6	183	283	52	96	0.98
30	15	73.5	8.9	15.9	4.5	13.3	170	312	46	103	0.60
Aug. 20	22	61.0	5.5	10.8	2.9	12.5	138	277	56	64	
Sept. 10	22	70.5	6.0	12.8	2.8	13.0	117	202	35	95	0.52
30	24	59.7	4.6	8.8	2.4	-	141	201	43	-	0.41

Table 10.

First year Danish cocksfoot (Hay type).

Date	Average Height inches	Bulk Moisture (%)	Crude Protein (%)			Carotene (Mg./kg. dry wt.)			Ratio Leaf/stem (dry wt.)
			Whole	Leaf	Stem	Whole	Leaf	Stem	
July 28	13	80.2	12.4	13.4	6.6	199	189	80	4.05
Aug. 5	15	73.0	10.7	13.1	6.0	270	375	76	3.73
29	18	72.2	8.2	9.7	5.2	155	246	31	2.38
Sept.17	21	72.2	7.6	8.5	4.1	176	256	36	2.17
Oct. 7	23	76.6	7.7	9.8	4.5	191	285	34	2.10
Nov. 10	23	78.6	5.7	6.6	4.2	143	319	40	1.83

FIG 6

LEAF-STEM RATIO IN 1ST YEAR GRASSES

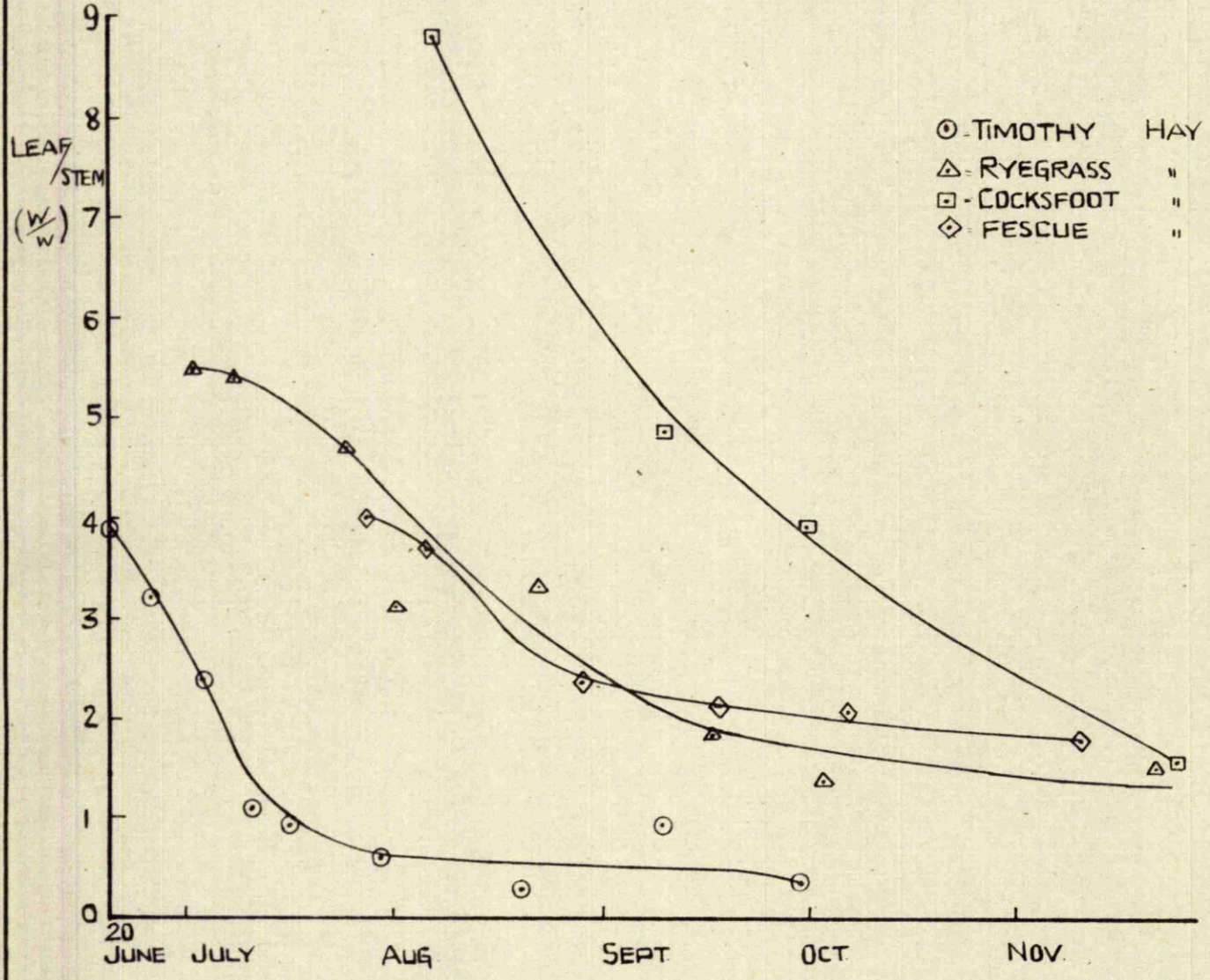


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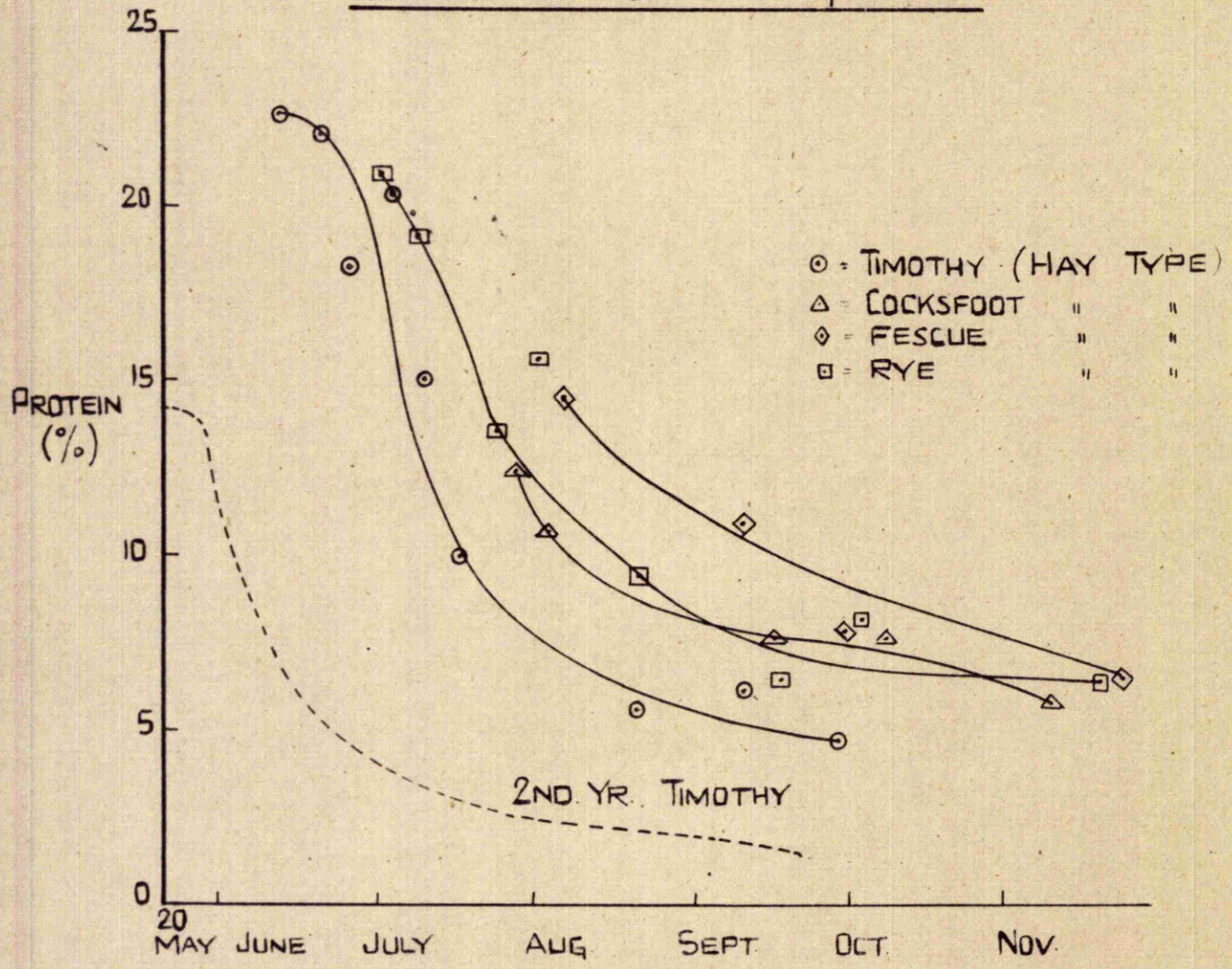
FIG 7

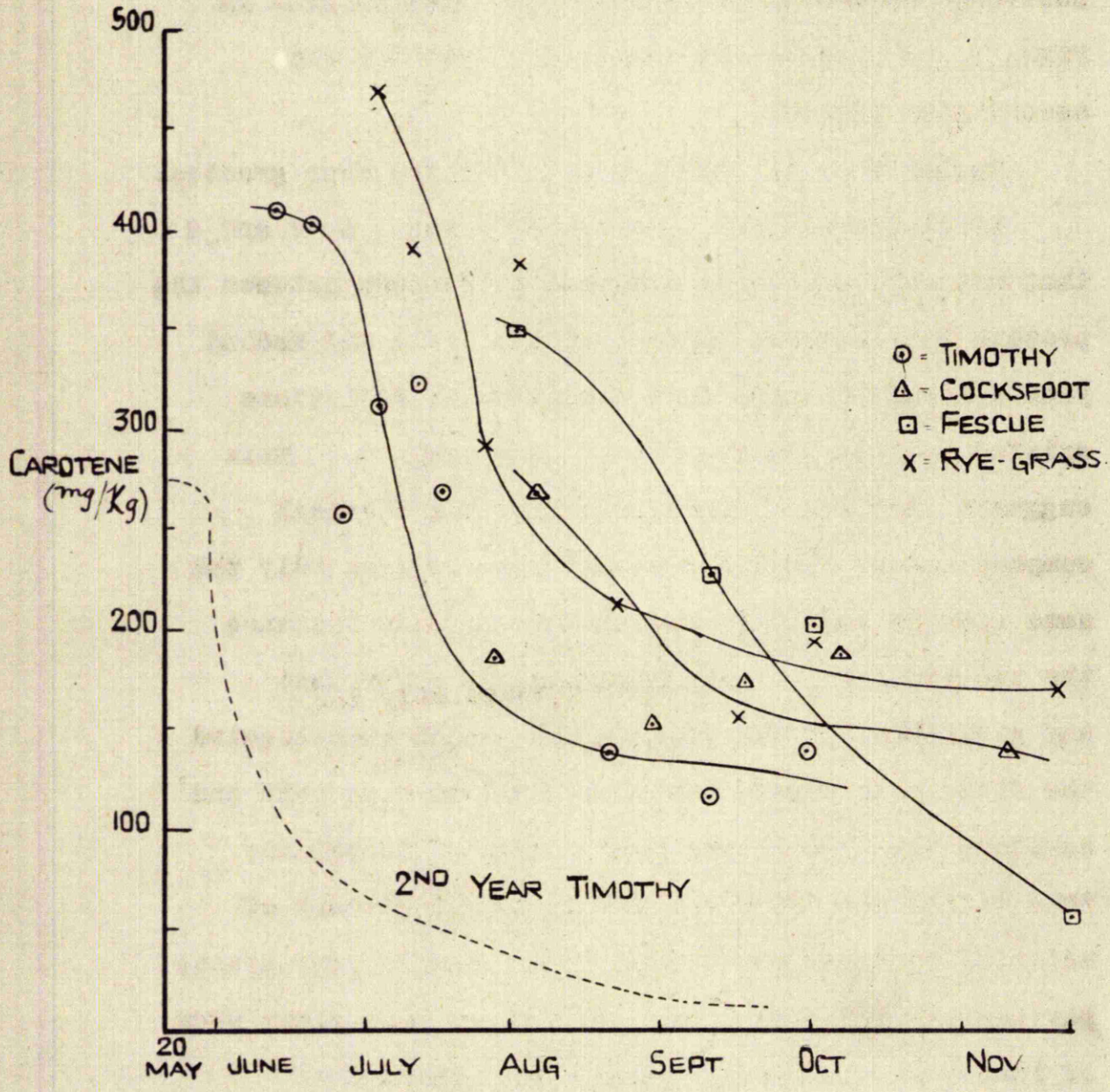
THE EFFECT OF SEASON ON THE PROTEIN CONTENT OF 1ST YEAR GRASSES.



SEASON 1947

FIG. 8.

THE EFFECT OF SEASON ON THE CAROTENE CONTENT OF 1ST YEAR GRASSES



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sufficient points were obtained to give the general shape of the curves in Figs. 6, 7 and 8. These illustrate the changes with the season in the ratio of leaf to stem, and in the contents of protein and carotene for the whole plants. The dotted line in Figs. 7 and 8 shows the comparable data for the second year timothy.

Tables 9 to 12 give the data for the four grasses.

It is immediately apparent from Figs. 6, 7 and 8 that not only was there a marked difference between the protein and carotene content of the first and second year grasses but also that considerable variations existed between the four first year grasses. This suggests that for any comparison of the chemical composition of first and second year grasses only the same species should be considered, in this instance the two timothys. Such a comparison shows that at any given time of the year in the period investigated the first year plants contained much more protein and carotene than the second year plants. To balance this difference there was the much greater bulk of material from any given area of the second year grass, particularly May and June. The first year plots were of insufficient size to provide any useful data concerning yield of dry matter but from other work (26) it would appear that over the whole growing season the yields from barley in its first and second year, under the same conditions of management are roughly in the

Aug. 7	9	86.2	14.7	17.0	8.5	350	474	80	885
Sept.10	15	75.0	10.9	13.1	5.3	228	325	35	4.85
Oct. 1	19	76.7	7.8	9.2	4.1	204	309	58	5.94
Nov. 24	22	80.3	6.5	8.0	2.9	57	127	14	1.56

[redacted]

Table 12.

First year Italian ryegrass (Hay type).

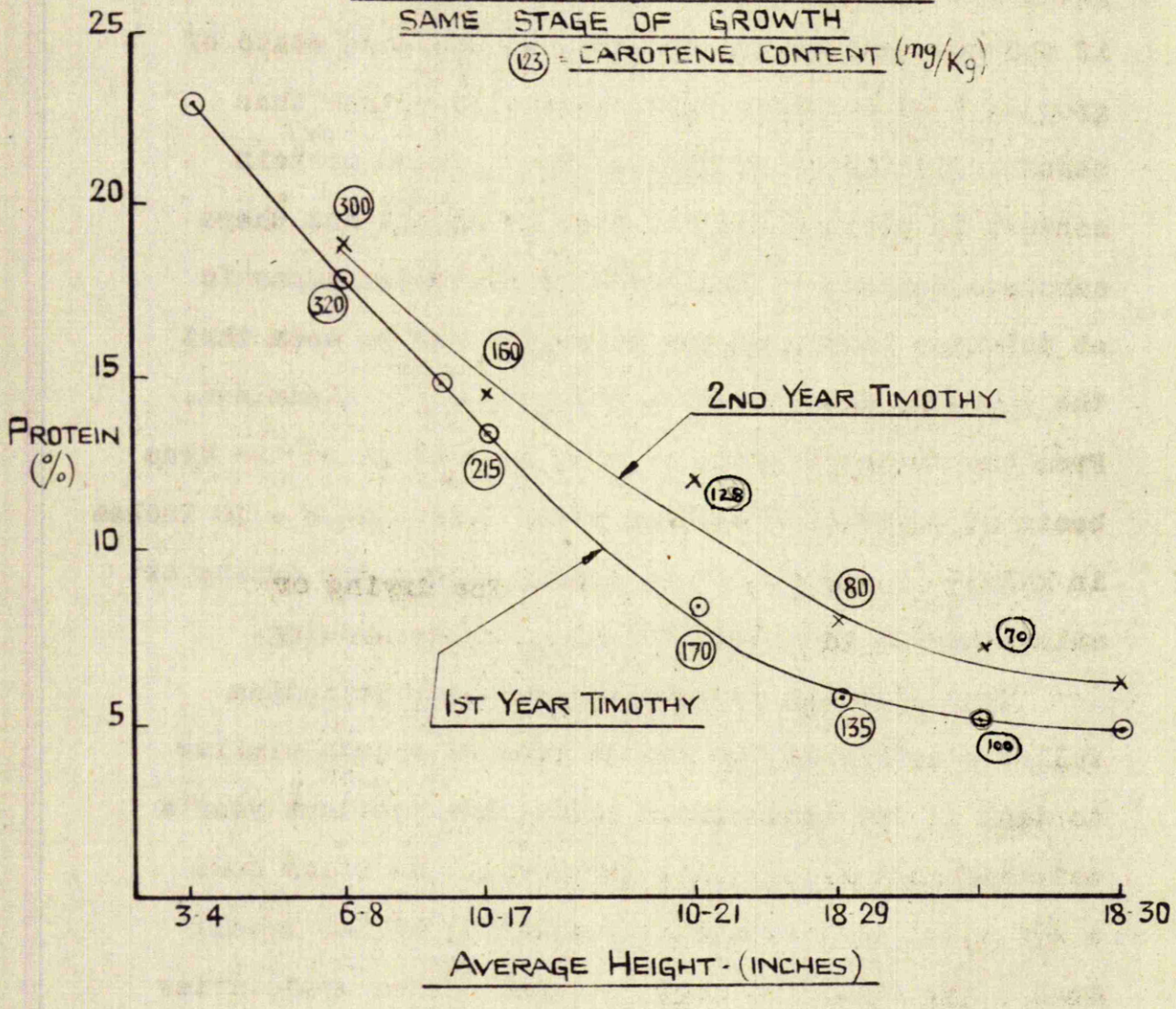
Date	Average Height inches	Bulk Moisture (%)	Crude Protein (%)			Carotene (mg./kg. dry wt.)			Ratio Leaf/Stem (dry wt.)
			Whole	Leaf	Stem	Whole	Leaf	Stem	
July 2	5.5	85.1	20.9	22.9	13.6	470	566	94	5.55
9	6	77.3	19.1	19.9	10.8	393	415	104	5.52
25	8	84.0	13.5	17.1	6.8	293	402	44	4.70
Aug. 1	12	86.7	15.5	16.5	7.6	385	462	14	5.10
22	14	61.4	9.3	10.8	4.8	214	277	41	3.33
Sept.17	14	85.5	6.4	6.6	4.6	160	226	41	2.20
Oct. 3	15	72.6	8.0	8.4	6.2	195	209	45	1.37
Nov. 21	16	86.2	6.4	7.3	5.8	109	171	14	1.53

[redacted]

FIG 9.

1ST & 2ND YEAR TIMOTHY AT THE SAME STAGE OF GROWTH

(123) = CAROTENE CONTENT (mg/Kg)

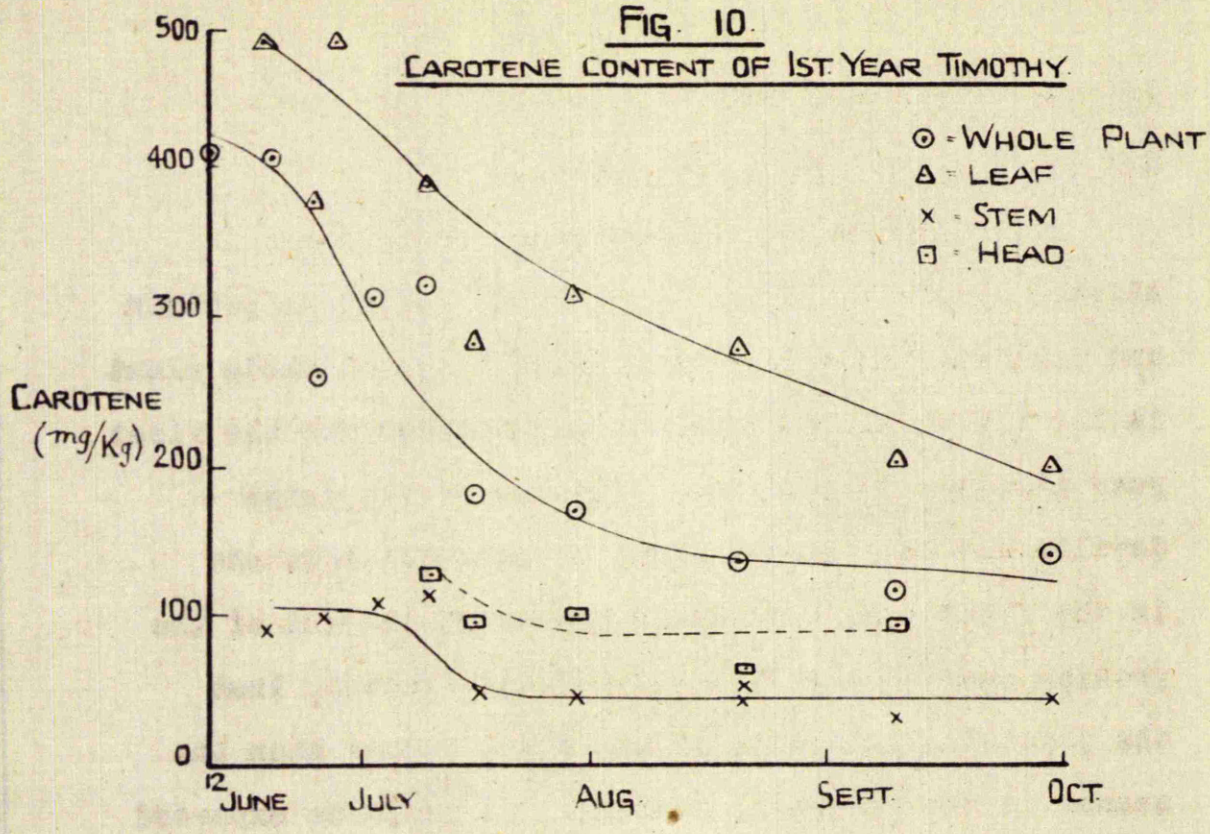


proportion of 5 to 6. To make up for the lower protein value the second year grass would need to yield at least twice as much as the younger grass. It should be realised, however, that when the very high carotene and protein contents exist in the first year grasses the plants are only 3 to 4 inches in height. Moreover, if the two grasses are compared at the same state of growth, i.e. compared physiologically rather than chronologically as in Fig. 9, where crude protein content is plotted against average height and where carotene content is indicated by encircled numerals at selected points on the curve, it can be seen that the apparent differences are considerably minimised. From the farmer's point of view this would be the true basis of comparison because grass less than 8 - 10 inches in height is uneconomic to handle either for drying or silage making and would not stand heavy grazing.

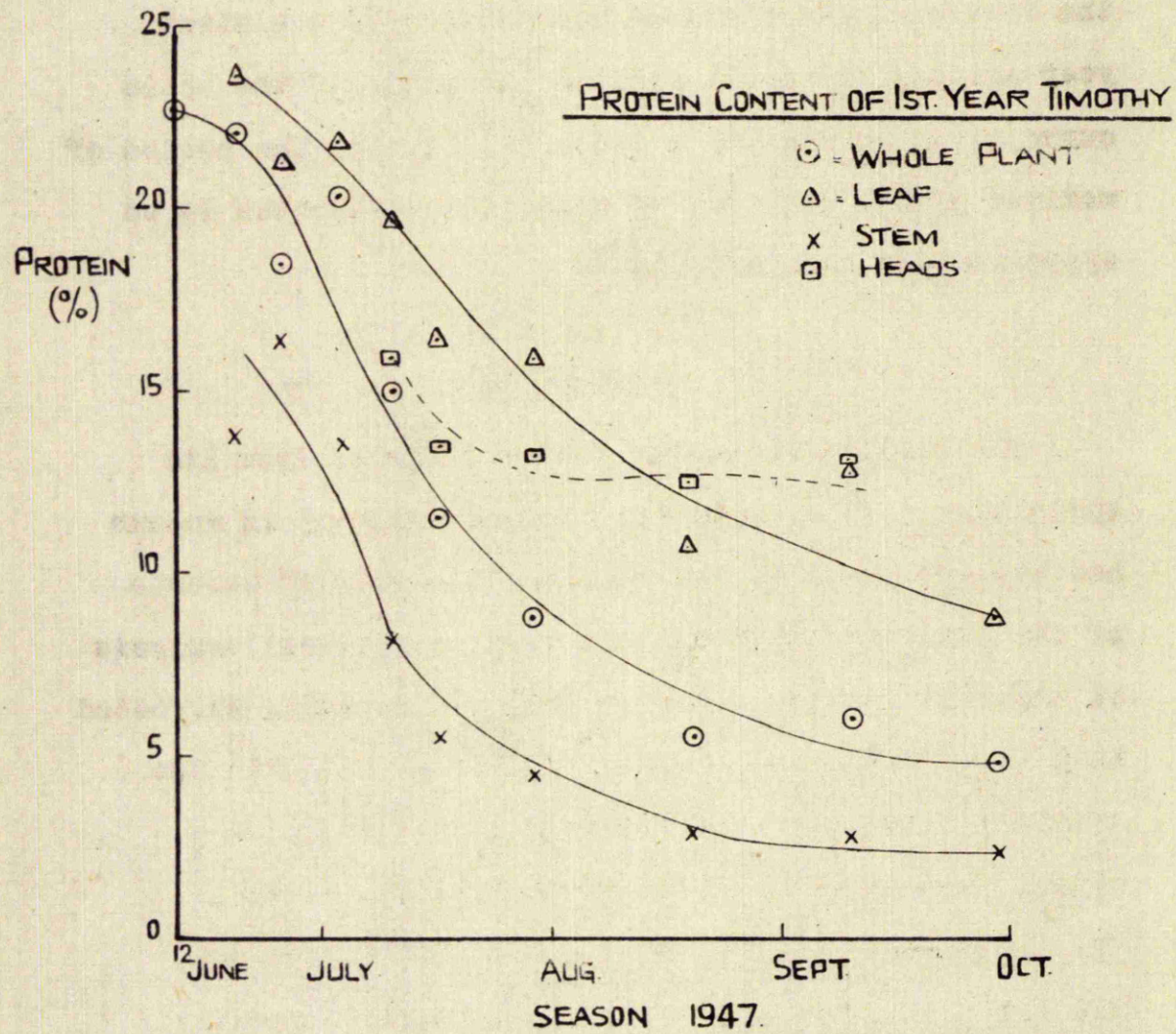
Thus although protein and carotene formation follow a course in the second year of growth similar to that of the newly grown plant, the previous year's establishment allows this development to occur some 6 - 7 weeks earlier and at a slightly higher level. Such a gap should be most grateful to the grass drier whose aim is to extend the limited period when his grass is of first quality over as great a part of the year as possible. Moreover, the differences in composition between the four types noted here would aid such a scheme, in this instance the fescue being a

FIG. 10.

CAROTENE CONTENT OF 1ST YEAR TIMOTHY.



PROTEIN CONTENT OF 1ST YEAR TIMOTHY



SEASON 1947.

further six weeks behind the timothy, with ryegrass and cocksfoot lying intermediately.

Quantitative differences similar to those already noted in the 2nd year timothy exist in protein and carotene content between leaf, stem and whole plant in the first year grasses, as illustrated for the first year timothy, in Fig. 10. Because of its later development the general level of both constituents in the first year grasses is higher at the end of the growing year than in the older grass, showing that the physiological state of the plant rather than the season is the governing factor. As would be expected the leaf/stem ratio starts much higher in the first year grasses but again follows the shape of the basic curve given in Fig. 2, a steep fall during the period of maximum growth followed by a very slow decrease to an approximately constant level.

DISCUSSION.

The familiar seasonal change of grass from its springtime leafiness to its fibrous maturity in autumn has been followed by the chemical analysis of certain of the plant constituents and also by physical analysis of the plant parts. Watson (58) has recently suggested that three distinct steps can be associated with the season's growth. These are (a) the early stage; plants leafy, little stem and no flowering heads, (b) the early flowering stage; some flowering heads but not in all types of grass, (c) the full flower

Table 13.

The analyses of first and second year timothy at three different stages in its growth.

	Early stage		Early flowering stage		Full flower stage	
	2nd year	1st year	2nd year	1st year	2nd year	1st year
Leaf-stem ratio	2.2	2.4	0.6	0.6	0.2	0.4
Carotene (mg./kg. dry wt.)	173	312	148	170	42	41
Protein (%)	18.8	20.3	9.2	8.9	3.9	4.6
Fibre* (%)	22.6	-	31.2	-	35.8	-

* Calculated from individual values for leaf and stem and the leaf/stem ratio.

stage when all seed is set or setting. Applying this classification to the 1st and 2nd year timothy grasses furnished the results shown in Table 13.

It can be seen that in the early stage the grass would be most suitable for conserving as a concentrate either in the form of silage or as dried grass. The early flowering stage would provide a good hay type of grass and one often better than normally produced, whilst grass which has reached the third stage would have only poor feeding qualities. From the shape of the curves in Figs. 6 and 7 it is apparent that the period over which grass grown for its high protein-low fibre composition can be obtained is very short. Indeed, adverse weather, such as lack of rain coupled with a high temperature in the early part of the year may cause the grass to "shoot", forming flowering heads on short stems with less than the normal complement of leaves. Such grass would then fall into the second class much earlier in the year than it ought. The effect of regular cutting at the early stage will be dealt with in Section 5.

Fagan and Milton (14) found that when second year timothy was cut at the "pasture" stage, about 4" high, the leaf-stem ratio was 5.7 with 11.1% protein but when the same grass was cut for hay the leaf-stem ratio had fallen to 1.1 and the protein to 6.8%. Fagan, Milton and Provan (15) in a classic investigation of the effect of applications of sodium nitrate to ryegrass

and clover, separated leaf and stem and found very similar seasonal changes for these plants as those recorded for timothy grass. They investigated also the effect of weekly and monthly cutting and as would be expected, the short leafy herbage of the weekly cuts has considerably higher protein and less fibre than that cut monthly. With attention turning later to carotene content, other workers have noted the decrease in chlorophyll and carotene with advancing age of the grass. Watson (55) and Maynard (34) have shown that young plants are rich in carotene while mature ones contain negligible amounts. Stanley (48) reports that early cut grass to be twice as potent as mature grass and 100 times as much as November cuttings with respect to Vitamin A. Similar conclusions have been arrived at by Thomas and Elliott (50). Virtanen (51) observed the same trend in oats and barley and suggested that the predominating factor in determining both carotene and protein was stage of growth.

The data in Table 3 and 4 and Table 10 show clearly that the reason for the high protein and carotene at the early stage is the preponderance of leaf over stem. At all times of the year the leaves contained more protein, more carotene and less fibre than the other parts of the plant, hence young leafy grass is essential for concentrate production. Even grass intended for hay, if allowed to grow past the early flowering stage in an endeavour to gain in bulk willbe low in protein and carotene and high in fibre.

Fagan (11) showed that postponing the cutting date by seventeen days, from June 22nd to July 9th, lowered the crude protein of a mixed ryegrass-clover growth from 10.8 to 6.7% with an increase in fibre from 28 to 32%. Similarly Crampton and Forshaw (7) found that the feeding value of meadow grass and brome grass changed throughout the year and that spring herbage was the best, partly on account of its high protein content and partly because of the greater availability of the carbohydrate fractions. With first year grasses a little more latitude may be allowed in the time of cutting because the leafy stage persists longer during the period of the plants establishment, a fact which should be useful in planning grassland for conservation.

It has been mentioned that it was hoped that analysis of the data of the separated grasses would answer the question whether it was worth while to endeavour to build a machine to perform this operation mechanically. Although the grasses on which leaf-stem separation was carried out received no fertiliser in the year under investigation, they were grown on land in good heart and would not be unduly worse than grass grown on many British farms. From Part 3 of this thesis, it will be found that heavy fertiliser applications can considerably increase the protein content of grass but it is unlikely that the general trend of protein levels of leaf and the whole plant with respect to each other over the season, will be

markedly different from those of the grasses investigated. To make the separation of leaf from stem economically worth while would require a minimum protein difference of five percent in favour of the leaves as compared with the whole plant. If this assumption is accepted, it can be seen from Figs. 5 and 10 that these conditions occurred in the second year timothy over the 10 weeks period June 17th to August 25th and in the first year timothy over the 12 week period from July 20th to the end of September. But only in the first two weeks of these periods would the leaf contain more than 14% crude protein which is actually 3% below the minimum to be considered as a protein-rich meal of first grade. Holmes (26) has shown that the application of heavy dressings of nitrogenous fertiliser can produce grass with crude protein contents as high as 26-30% at a height of 8-10 inches and it seems reasonable to assume that the protein content, if such a grass was allowed to grow to maturity, would follow a similar downward curve to that of the grasses investigated. If that were so, the length of time over which the leaf would contain 5% more crude protein than the whole plant and also contain not less than 16-18% crude protein would be about 5 weeks. This increase in time might be useful were it not for the fact that by proper management, such as provision of first and second year leys of different species as suggested above, together with the liberal application of nitrogenous dressings, an equal if not better

spread-over of first grade grass for drying could be accomplished, and grass which had passed the "concentrate" stage could then be utilised for the equally necessary hay.

The conclusion must be, that on the data presented, methods of grassland management are possible which render unnecessary the difficult separation of ageing grass into leaf and stem fractions.

PART 3.

THE EFFECT OF MANURIAL TREATMENT ON THE COMPOSITION OF GRASS.

The work described in this section formed part of the grassland investigation programme of the Hannah Institute and was planned and conducted largely by Dr. W. Holmes. The author was concerned mainly with the estimation of carotene in the grasses and also to a lesser extent with the determination of crude protein. The following account deals only with these two grass constituents but to complete the picture, the other aspects of the whole experiment, such as dry matter yields, mineral constituents etc. are briefly recounted in Appendix 1.

The results already discussed in Part 2 have shown that grasses quickly pass from a nutritionally valuable state to one where their best use can be nothing more than as a bulky supplement in the maintenance ration for dairy cattle. Equally, the compositional changes of different species were shown to occur at varying rates, and in an experiment which included manurial treatments likely to affect the growth rates considerably, it was obvious that cutting the grass on a fixed chronological basis would be useless. It was decided that the physiological state of the herbage should be the determining factor and all cuts were taken when the grass was in the young, leafy stage prior to heading which, in the season under

review (1946) usually occurred at a height of eight to eleven inches. This gave a product of high nutritional value of the type most suitable for conservation either by drying or ensiling.

Two pastures were used, in Series 3 a one year ley of Italian ryegrass and broad red clover which had been sown out under barley in 1945 and in Series 4, a four year old, long ley in which ryegrass was predominant. The Series 4 pasture had been grazed by a dairy herd for the previous three years.

Plot size.

It had previously been found that plots 1/200 acre in area were a suitable size as long as three replicates for each treatment were employed. Nine manurial treatments were investigated, hence each series consisted of three blocks of nine plots arranged so that each treatment appeared in each block and each row.

Manurial treatment.

Heavy applications of a nitrogenous fertiliser were to be employed and as ammonium sulphate is said by Lewis (32) to be harmful to crops and soil in large amounts, nitro chalk was used as the source of nitrogen. Lime, basic slag or superphosphate and potassium chloride were used in the basic dressings and when phosphate and potassium were applied during the season a compound fertiliser (I.C.I.C.C.F. No. 1) containing 12% N, 12% P_2O_5 and 15% K_2O was used.

The basic dressings were as follows:

All plots: Lime, 1 ton ground limestone per acre.

Series 3: Superphosphate (18% P_2O_5) 5 cwt. per acre. Potassium (60% K_2O) ½ cwt. potassium chloride per acre.

Series 4: Basic Slag (18% P_2O_5) 5 cwt. per acre.

The nine experimental dressings were:-

Treatment A: 18 cwt./acre nitrochalk, 6 cwt. applied in March, 6 cwt. in May and 6 cwt. in July.

Treatment B: 12 cwt./acre nitrochalk, 6 cwt. applied in March, and 6 cwt. applied in May.

Treatment C: 6 cwt./acre nitrochalk, 6 cwt. applied in March.

Treatment D: Control, no application.

Treatment E: 2 cwt./acre nitrochalk in March and after each cut.

Treatment F: 1 cwt./acre nitrochalk in March and after each cut.

Treatment G: 12 cwt./acre nitrochalk, 2 cwt. in March, 6 cwt. in May and 4 cwt. in July.

Treatment H: 6 cwt./acre nitrochalk, 2 cwt. in March, 2 cwt. in May and 2 cwt. in July.

Treatment I: 20 cwt./acre of a mixture of equal parts of C.C.F. and nitrochalk, 6.66 cwt. in March, 6.66 cwt. in May and 6.66 cwt. in July.

Table 14.

Summary of cutting data.

Treatment	Series 3		Series 4	
	Total number of cuts.	Av. height at cutting.(in.)	Total number of cuts.	Av. height at cutting(in.)
A	8	10½	5	10½
B	7	10½	5	10
C	7	10	4	9
D	5	10	4	9
E	8	10½	5	9½
F	7	10½	4	10
G	6	10	5	10
H	6	10	4	10
I	7	10½	5	10

Treatments A,B,C and D provided a comparison of the effect of different amounts of the same fertiliser. Treatments E and F used the same fertiliser as in A,B,C and D but applied as a number of light dressings. The object in Treatment G was to maintain production of protein in the natural seeding period by applying the heaviest dressing in May and can be compared with Treatment B which used the same quantity in a different manner. Treatments C and H were similarly comparable. In Treatment I the nitrogen was supplemented by phosphorus and potassium in the approximate proportions occurring in grasses and could be compared with Treatment A.

Sampling.

Four areas of 72 sq.in. in each plot were selected by random casts of a rectangular frame and the bulked grass used immediately for moisture, carotene and protein analysis.

Weather.

Until mid-June the growing season of 1946 was retarded by drought and cold weather, thereafter both rainfall and temperature were favourable for growth.

Cutting.

Table 14 summarises the effect of manurial treatment on the number of times plots subjected to the various treatments were cut during the season. These data are taken from the thesis by Holmes (26).

Table 15.

SERIES 3. One year ley. Crude protein and carotene contents.

P = crude protein %, C = carotene (mg./kg. dry wt.)

Treatment		A		B		C		D		E		F		G		H		I	
Cut no.		P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C	P	C
1st	Date	11/4		11/4		11/4		9/5		17/4		26/4		18/4		10/4		10/4	
		31.3	374	31.2	412	30.3	339	11.2	272	18.7	272	9.1	208	17.5	352	19.4	312	27.9	350
		30.9	382	28.6	408	30.1	377	10.9	276	16.3	276	13.1	235	17.4	336	19.2	299	30.9	419
		30.6	371	27.6	408	31.8	377	11.7	269	21.4	269	12.6	243	16.7	342	19.1	395	27.5	355
	Mean	30.9	376	29.2	409	30.7	364	11.3	272	18.8	272	11.6	228	17.2	343	19.2	334	286	375
2nd	Date	8/5		7/5		8/5		9/7		17/5		28/5		29/5		29/5		7/5	
		16.1	318	16.8	338	12.9	285	14.4	320	12.2	252	9.8	191	8.9	170	8.0	173	17.1	195
		16.0	329	16.8	358	15.6	274	12.8	263	12.0	245	11.5	196	9.6	188	8.0	172	17.1	209
		16.6	332	16.9	363	16.9	240	11.7	305	13.1	256	13.6	216	10.4	215	8.6	185	16.4	196
	Mean	16.2	326	16.8	353	13.1	266	12.9	296	12.4	251	11.6	201	9.6	191	8.2	177	16.9	200
3rd	Date	7/6		11/6		11/6		9/7		19/6		2/7		5/7		2/7		7/6	
		25.1	431	20.5	298	10.6	250	14.4	320	13.3	311	11.6	299	18.4	395	11.2	259	26.5	329
		25.3	424	22.3	311	11.1	288	12.8	263	13.0	304	15.6	283	18.9	448	10.9	280	22.4	292
		25.9	447	22.4	336	10.7	246	13.4	305	12.6	313	10.8	307	18.4	435	14.0	324	23.1	302
	Mean	25.4	434	21.7	315	10.8	261	13.5	296	12.9	310	12.6	296	18.6	426	12.0	288	24.0	308
4th	Date	4/7		5/7		8/7		2/8		8/7		23/7		25/7		25/7		4/7	
		19.6	448	15.5	316	12.3	360	19.9	462	17.3	473	15.3	355	14.5	426	16.9	363	17.1	423
		17.5	454	17.5	340	12.2	383	19.7	484	16.5	439	16.7	377	17.0	446	17.0	350	16.2	463
		17.9	476	19.0	350	13.7	339	19.5	455	16.3	401	15.8	363	19.0	430	20.0	385	16.7	429
	Mean	18.3	459	17.3	335	12.7	361	19.7	467	16.7	437	159	365	16.8	432	17.9	366	16.7	438
5th	Date	29/7		1/8		1/8		4/9		29/7		20/8		20/8		2/10		25/7	
		20.5	465	17.0	449	18.7	472	21.1	444	22.9	423	17.5	405	23.4	480	20.4	428	19.7	473
		19.8	454	14.9	388	18.3	485	19.8	401	20.0	456	13.0	342	22.7	435	20.1	420	17.1	463
		19.4	473	14.5	423	15.2	442	23.9	410	18.4	457	15.0	281	24.3	470	19.4	460	19.4	450
	Mean	19.9	464	15.5	420	17.4	466	216	418	204	445	15.2	343	23.5	462	20.0	436	18.7	462
6th	Date	29/8		4/9		4/9				23/8		25/9						16/8	
		23.3	485	17.1	433	21.0				19.6	423	22.1	490					20.7	505
		21.3	469	14.3	386	20.6				17.6	410	21.7	542					23.0	486
		23.4	527	16.2	418	18.8				20.6	456	22.1	469					21.6	465
	Mean	22.7	494	15.8	412	20.1				19.3	430	21.9	500					21.8	485
7th	Date	2/10		17/10		17/10				25/9		29/10						1/10	
		26.7	511	21.6	438	21.7	466			25.0	473	24.1	448					23.1	416
		24.9	512	19.6	447	21.6	542			24.7	511	24.1	467					21.1	392
		27.2	540	19.6	408	19.7	470			25.6	539	23.5	442					23.6	427
	Mean	26.3	521	20.3	431	21.0	493			25.1	508	23.9	452					22.6	412
8th	Date	30/10								29/10									
		26.4	401							26.4	468								
		22.8	456							27.6	515								
		25.4	409							27.2	479								
	Mean	24.9	422							27.0	487								

[redacted]

Table 16.

SERIES 4. Semi permanent pasture. Crude protein and carot

P = crude protein %, C= carotene (mg./kg. dry wt.)

Treatment		A		B		C		D		E		F		
Cut		P	C	P	C	P	C	P	C	P	C	P	C	P
1st	Date	14/5		14/5		14/5		22/5		23/5		21/5		2
		20.0	438	21.2	381	20.3	332	12.0	201	14.0	238	15.2	320	15.3
		17.6	422	20.4	356	16.6	326	11.6	215	17.4	224	14.1	295	15.0
		17.9	389	21.0	379	20.9	339	16.2	267	12.8	231	14.5	289	12.3
	Mean	18.5	417	20.9	372	19.3	332	13.3	228	14.8	231	14.6	301	14.2
2nd	Date	17/6		17/6		20/6		19/6		19/6		20/6		1
		24.4	484	24.5	499	14.6	329	11.9	256	15.0	266	15.7	353	20.5
		22.2	441	26.8	491	13.9	300	12.7	258	13.2	248	15.5	310	21.8
		26.9	524	25.8	449	15.7	305	14.4	325	12.3	280	14.9	335	21.8
	Mean	24.5	483	25.7	479	14.7	311	13.0	280	13.5	265	15.4	333	21.4
3rd	Date	18/7		18/7		2/8		12/8		23/7		26/7		1
		13.3	431	17.3	461	13.6	530	14.3	336	16.9	383	13.7	498	16.1
		17.0	433	17.5	475	16.7	551	14.9	315	19.6	440	13.3	524	15.6
		17.0	470	19.9	496	14.3	578	13.7	347	17.1	413	15.3	504	15.8
	Mean	15.8	445	18.2	474	14.9	553	14.3	332	17.9	414	14.1	508	15.7
4th	Date	16/8		5/9		15/10		16/10		5/9		20/9		
		23.4	518	16.2	604	19.1	416	16.8	455	16.0	454	19.1	505	20.
		26.8	587	15.2	538	17.2	478	18.2	462	17.1	513	20.6	525	19.
		21.3	540	15.3	544	18.4	418	18.0	471	18.0	559	17.1	477	17.
	Mean	23.8	548	15.6	562	18.2	437	17.7	463	17.0	508	18.9	502	19.
5th	Date	3/10		30/10						15/10				
		21.9	628	19.4	411					22.7	448			22.
		25.8	619	21.5	417					24.9	463			21.
		26.3	576	20.5	412					25.3	499			21.
	Mean	24.7	607	20.5	413					24.3	470			21.

Crude protein and carotene.

Tables 15 and 16 present the percentage of crude protein and the carotene content of the grass at each cutting, Table 15 refers to Series 3 and Table 16 to Series 4. Each figure is the mean of three separate samples taken from triplicate plots. The protein data is taken from the thesis by Holmes (26).

DISCUSSION.

In the foregoing experiments a study has been made of the extent to which the carotene and protein contents of grass at a highly nutritious stage could be increased by varying amounts of fertiliser applied in different ways. Comparison with the control plots showed the beneficial effect of the fertiliser in the increased number of cuts which could be taken over the same period and the increased amounts of carotene and protein in the treated grasses. This effect, as might be expected, was less marked in the older established grass which had been grazed for four years previously and in which the proportion of early growing strains would have diminished. The quicker growing young ley was ready for cutting a month before the pasture plots and this must be attributed mainly to growth habits rather than response to applied nitrogen. Although the plots in both fields received, for any given treatment, the same weight of nitrochalk per acre it is likely that the density of the plants

in the two series was considerably different. The soil bearing the ryegrass and clover which had been undersown with oats in the previous year would contain many less roots than that of the established grass pasture and, being unencumbered with a thick sward would allow quicker penetration of the fertiliser and would provide a greater concentration of available fertiliser per plant. In addition, the first year ley would still contain the early growing strains. The effect of increasing the first fertiliser dressing, from 1 to 6 cwt./acre was to decrease the time interval between application and cutting from 37 to 28 days in Series 3 and from 62 to 55 days in Series 4. Earlier cutting following application of 23.2 and 69.6 lbs./acre of $(NH_4)_2SO_4$ and 46.4 lbs./acre $Ca(NO_3)_2$ respectively was also found by Blackman (5) although not to quite the same degree.

(a) Crude Protein.

Although the physiological state of the grass was sensibly the same at each cut, the crude protein content of all plots varied considerably from one cut to another. In Series 3, in all except the control plot and Treatment F (1 cwt. nitrochalk after each cut) the protein content was highest in the spring and autumn. Treatment A (18 cwt./acre nitrochalk in three equal dressings) resulted in the highest average protein content, and a small number of heavy spring dressings resulted in a greater

protein content than the same weight applied more regularly but in smaller quantities over a longer period. Addition of potassium and phosphorus gave a very similar protein content throughout the season to the same nitrogen treatment without potassium and phosphorus and did little to prevent the midseason drop referred to by McNair & Fowler (35). Archibald (1) and Watson (60) who used lighter dressings both found a similar trend in the summer months.

In Series 4 the protein contents of the semi-permanent pasture followed very much the same pattern as in the temporary ley although at a slightly lower level and again, the highest values were recorded in spring and autumn.

(b) Carotene.

From Tables 15 and 16 it can be seen that in both series the carotene content followed closely the general trend of the protein content. It was not, on the whole, subject to such wide fluctuations as the protein content and some of the carotene values may appear rather high. It should be remembered that during this part of the work the analytical method employed in the separation of carotene from the fresh grass was one known to give erroneously high results. This matter was fully discussed in Part I.

The effect of increased amounts of nitrogenous

FIG. 11.

CORRELATION BETWEEN PROTEIN & CAROTENE OF GRASS IN SERIES 3.

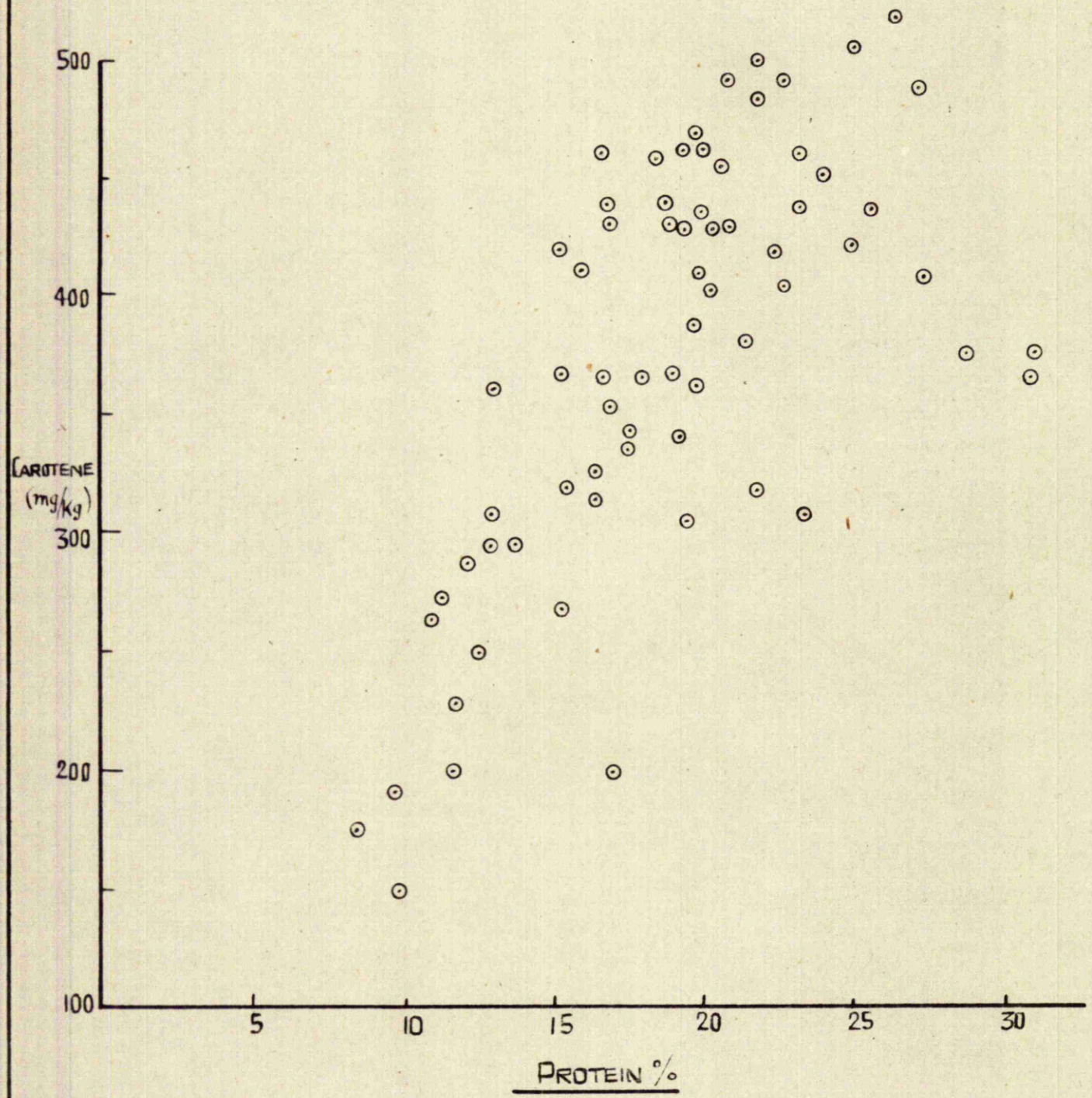
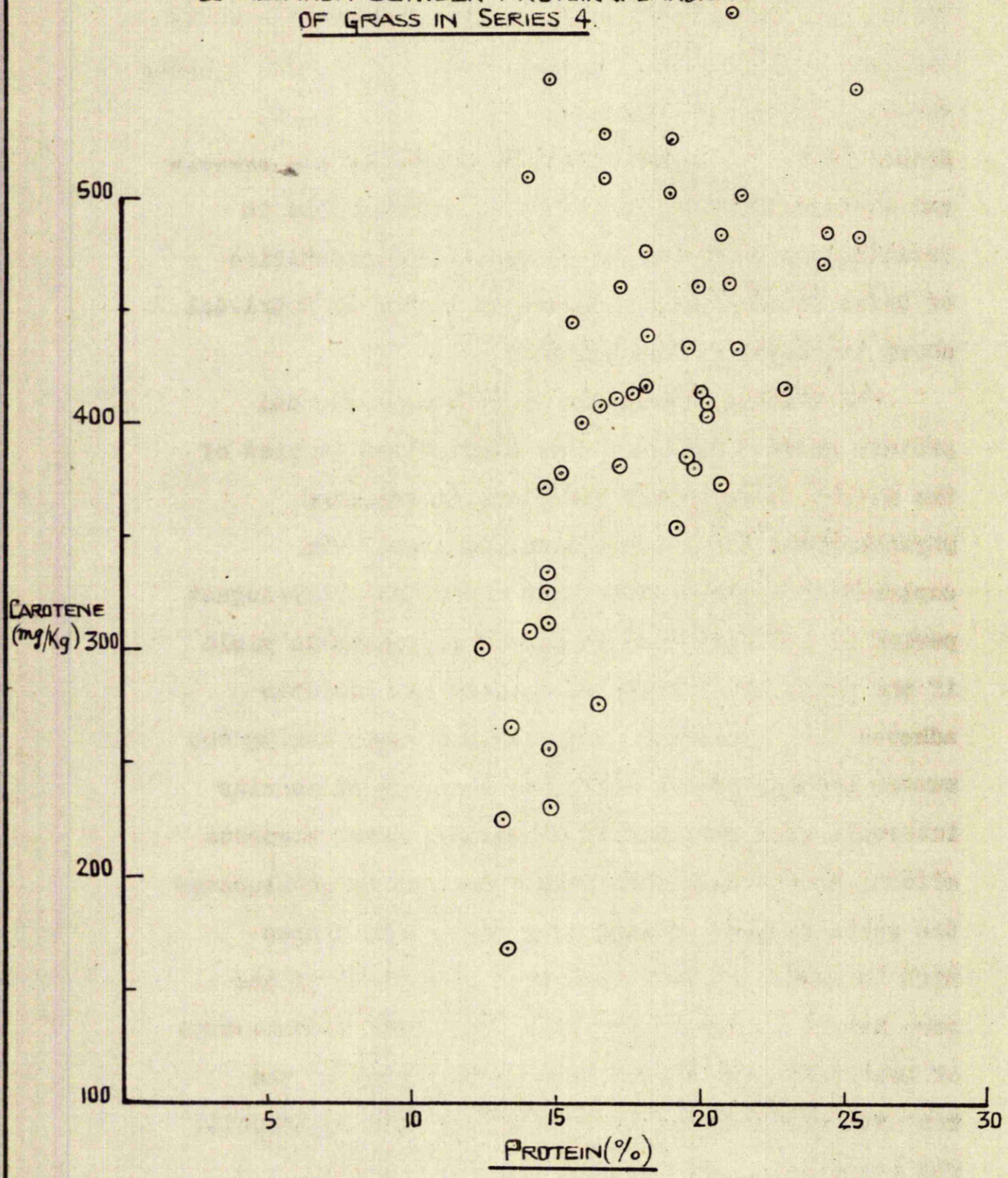


FIG. 12

CORRELATION BETWEEN PROTEIN & CAROTENE OF GRASS IN SERIES 4.



fertiliser (Treatments A to D) resulted in the highest carotene content in the grass receiving most fertiliser, with the control plot containing least. Direct proportionality between fertiliser applied and protein and carotene content is not to be expected and in Treatments A to C a ratio of fertiliser quantities of 6:2:1 is reflected in carotene ratios of 2.4:1.4:1 above the level of the control.

The seasonal variation in both carotene and protein content requires some elucidation in view of the attempt made to cut all plots at the same physiological stage throughout the year. The explanation appears to be that during the July-August period it was difficult to obtain a reasonable yield if the young leafy stage of cutting was strictly adhered to. This was most likely because during the summer months, grass, which has been cut at regular intervals each time before flowering, makes vigorous efforts to put up a flowering stem, and in consequence the ratio of leaf to stem of a plant 8-10 inches high in August is less than that of a plant of the same height in May or October. No direct measurements of leaf/stem ratios were made in this part of the work but direct observation confirms this hypothesis. The increase in the proportion of stem leads to a fall in both protein and carotene during the mid-summer months.

In Figs. 11 and 12 the values for crude protein

Table 17.

Correlation of crude protein and carotene.

Treatment	SERIES 3			SERIES 4		
	Corr.Coeff.	Carotene/Protein Ratio		Corr. Coeff.	Carotene/Protein ratio	
		Spring	Autumn		Spring	Autumn
A	+ 0.129 n.s.	20.1	16.0	+ 0.716 h.s.	22.5	24.6
B	+ 0.101 n.s.	15.1	21.3	-0.370 n.s.	17.9	20.5
C	+ 0.686 h.s.	17.7	23.9	+ 0.808 h.s.	17.3	24.0
D	+ 0.904 h.s.	15.2	18.7	+ 0.878 h.s.	17.1	26.1
E	+ 0.895 h.s.	19.5	20.2	+ 0.776 h.s.	15.6	19.3
F	+ 0.895 h.s.	19.7	18.9	+ 0.393 n.s.	15.6	26.5
G	+ 0.935 h.s.	19.8	21.7	+ 0.536 s.	21.7	25.6
H	+ 0.907 h.s.	15.8	21.9	+ 0.663 s.	23.0	22.1
I	+ 0.094 n.s.	13.1	17.9	+ 0.580 s.	19.3	23.1

n.s. = not significant, s = significant, h.s. = highly significant.

are plotted against those for carotene for all the cuts and treatments of Series 3 and 4. Again, as in the previous timothy experiments, the two constituents can be seen to be closely inter-related. Table 17 gives the correlation coefficients and shows that out of the eighteen correlations arising from the nine treatments in the two series, ten were highly significant, three significant and five not significant.

Included in Table 17 are the ratios of carotene to protein (obtained by dividing mg./kg. carotene by % crude protein) at the beginning and end of the growing season. In all except three of the treatments more carotene was associated with a given percentage of protein in the autumn than in the more rapidly growing spring grass. Moon (58) has reported similar results. This may be because even in this very leafy material the natural habit of producing a flower stem in the spring grass results in slightly stemmier material than late in the year when the shortening days preclude any liklihood of flowering.

In Series 4, carotene values were generally higher from the established sward than in the ryegrass temporary ley, particularly in spring and autumn.

In conclusion, it would appear that manurial

treatments likely to increase the yield of crude protein will, in general, increase the carotene content and that the ratio of carotene to protein is likely to be greater in autumnal growth. The greatest yield of both protein and carotene is to be expected from a small number of heavy spring dressings.

PART 4.

THE CAROTENE CONTENT OF DRIED GRASS.

The work in this section is concerned chiefly with the carotene of grass and a study of its loss during the three processes of preparation of the grass for drying, the drying process itself and the storage of the dried grass, with a view to reducing such losses to a minimum. As pro-vitamin A, β-carotene is of considerable value to animal health and as such is an important constituent of grass. It is, unfortunately particularly susceptible to destruction as was mentioned when discussing methods of analysis of this pigment. In freshly cut grass the carotene is immediately liable to oxidation, photochemical breakdown and enzyme action, to give products incapable of subsequent formation into material possessing vitamin efficiency.

(a) Carotene losses prior to drying.

In commercial grass drying any reduction in moisture content that can be produced in the fresh grass before drying is most desirable. Assuming a moisture content of 80% it is evident that in every 5 tons of fresh grass there will be four tons of water, whereas if the moisture content had been previously reduced to 67% the grass drier would need to evaporate only half that quantity. Such a reduction can be achieved by wilting the cut grass in the field for a period of some hours, the duration of which will depend on the weather conditions. The gain in case of

Table 18.

Effect of wilting on the carotene content of fresh grass.

Hours wilted	Moisture Content (%)	Carotene (mg./kg. dry wt.)	Carotene Loss (%)	WEATHER CONDITIONS								Crude Protein (%)
				Air temp. over 24 hr.			% rel. humidity at		Sunshine (hours)		Rainfall	
				Max. °F	Min. °F	Aver. °F	1000 hr.	1700 hr.	Daily	Cumulative	mm.	
0	77.6	214	0									12.7
2	65.9	186	13									12.2
4	55.9	175	18	86	34	75	61	50	10	10	Nil	12.4
8	41.5	157	27									12.7
24	43.3	126	41									11.8
27	39.2	85	60									12.1
30	33.5	78	63	86	42	75	45	43	12	22	Nil	12.0
48	33.1	74	65									12.0
51	31.2	38	82									11.7
54	28.2	32	85	82	30	72	47	48	12	34	Nil	12.2
72	31.5	29	86									11.9
78	27.9	31	86	76	42	69	55	51	12	46	Nil	12.3
96	24.3	28	87									12.0

drying will be obtained at the expense of the carotene content of the grass, and the following experiments were made to investigate the extent of such losses.

During a period of fine, warm weather in late May, grass of 10-15 inches length from a temporary ryegrass-cocksfoot ley was cut and allowed to wilt for four days. It was turned by hand forking every two hours during the first day and thereafter every three hours during the daylight period of successive days. A sample was taken immediately after cutting and prior to each turning. This was made as representative as possible by taking small quantities from points distributed equally over the cut area. From the bulk sample a smaller number of plants representative of the whole were taken for determination of carotene and crude protein contents. The remainder of the large sample was dried in a vertical oven at 110°C. to a constant weight to provide data from which to calculate the moisture content. The air temperature at ground level was recorded on a thermograph and the number of hours of direct sunshine to which the wilted grass was exposed noted by direct observation. Relative humidity was measured by means of a sling hygrometer twice daily at 1000 and 1700 hours B.S.T. Rainfall figures were obtained from the meteorological station at Auchincruive, 800 yards to the south of the experimental plots. The results are given in Table 18 and in Fig. 15 the carotene content is plotted

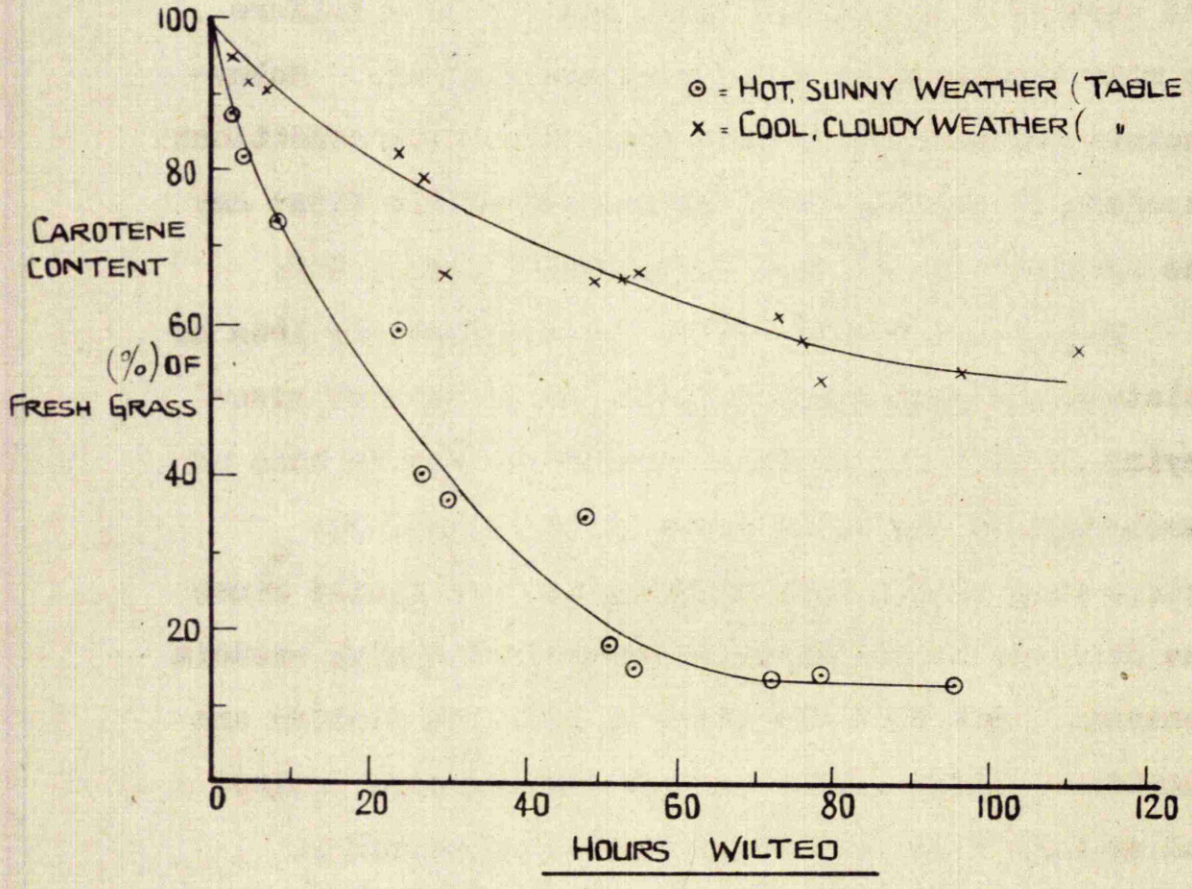
Table 19.

Effect of wilting on carotene content of fresh grass.

Hr. Wilted	Moisture (%)	Carotene (mg./kg. dry wt.)	Loss (%)	Temperature during 24 hr.			R.H.%		Sunshine hours.		Rainfall
				Max.	Min.	Av.	1000 hr.	1700 hr.	Daily	Cumulative	mm.
0	87.2	277	0								
2	85.4	263	5								
5	83.2	253	8	56	40	45	86	64	7	7	Nil
7	83.1	252	9								
24	79.3	229	17								
27	78.9	220	20	56	40	50	70	80	4	11	0.8
30	68.1	184	33								
49	87.1	186	34								
52	78.4	185	34	58	42	50	90	87	4	15	Nil
55	77.6	189	33								
73	83.4	169	39								
76	82.7	161	42	54	42	48	90	85	1	16	0.4
79	79.1	146	47								
97	76.7	139	50	55	39	45	87	85	6	22	Nil
122	76.0	160	51	51	37	43	87	86	2	24	Nil

FIG. 13

EFFECT OF WILTING ON CAROTENE CONTENT OF FRESH GRASS.



against the length of time for which the grass was wilted.

The farmer is often faced with an adverse change of weather soon after cutting grass, either for grass drying or, perhaps more often for haymaking, and Table 19 records data similar to that in Table 18 but under worse climatic conditions.

Soon after the start of this repeat experiment the weather worsened and there was complete failure to wilt the grass to a low moisture content. Nevertheless the data shows that even under such conditions carotene is rapidly lost, at the end of the first day the original content had decreased by nearly 25%.

From these results it is obvious that any loss of moisture and consequent gain to the economy of grass drying is obtained at considerable expense in loss of carotene. If the dried grass is to be used for cattle feed such a loss probably matters little since the chief value of the dried grass is its high protein content. But when the needs of pigs and poultry are considered their source of carotene are more limited and as high a content of carotene as possible is required in the dried grass. After taking into account possible carotene losses in dried grass from other causes it would appear that the loss in wilting should not exceed ten per cent of the original fresh value. From the curve correlating loss of carotene with evaporation of water for the first day it appears that

this amount of carotene loss accompanies the removal of 10-15% water from the freshly cut grass. This means that the permissible time of wilting will vary with the weather conditions and in sunny, warm weather 8 hours is perhaps the most that can be allowed if a high carotene grass meal is desired. In this length of time the moisture content may be expected to fall to about 70%.

(b) Carotene losses during drying.

There are many different commercial grass driers in use, employing a variety of systems of operation, but one type responsible for much of the grass dried in Britain consists of blowing hot air through a bed of grass travelling forward on a metal belt. Messrs. Petrie and McNaught have specialised in this system and a machine manufactured by this firm has been in use for a number of years at the Hannah Institute. The fresh, or wilted grass, is fed by hand on to a preliminary conveyor belt from which it is discharged on to an endless steel belt, 6½ feet in width. The steel belt carried the grass through a drying zone heated by gases from a coal-fired furnace; the temperature around the grass is kept at about 340°F. (170°C.) by admixtures of atmospheric air. Although the speed of the belt is adjustable and considerable variations in temperature can be obtained it was felt that a closer control of such factors was desirable to investigate their effect on the carotene content of the dried grass. To this end a small laboratory drier was constructed capable of dealing with about 500 g.

Table 20.

Comparison of Petrie-McNaught (P.M.) and Laboratory Grass Driers (Lab.)

Sample No.	Position on bed of P.M. (feed end)	Moisture Content (%)			Drying air temperature °C		Time in Drier (min.)		DRIED GRASS Carotene (mg./kg. dry wt.)			DRIED GRASS Crude Protein (%)
		Fresh	P.M.	Lab.	P.M.	Lab.	P.M.	Lab.	Fresh	P.M.	Lab.	
1	Left	85.1	7.59*	3.83	165	114	25	45	407	432	373	21.4
2	Right	85.0	4.26	3.49	162	114	32	45	416	408	393	22.6
3	Centre	84.0	3.12	2.93	170	111	33	45	413	396	400	25.5
4	Left	84.1	3.13	3.58	157	110	30	45	429	399	401	23.5
5	Right	84.5	4.10	3.42	180	113	34	45	451	389	379	23.2
6	Centre	84.8	3.54	4.49	170	110	32	45	455	391	406	26.4
7	Left	73.1	2.45	5.64	174	105	20	45	203	150	170	19.6
8	Right	70.0	3.86	3.88	171	105	20	45	209	212	182	20.6
9	Centre	70.0	4.76	4.31	171	105	20	45	240	209	193	19.6
10	Left	77.3	5.27	5.61	174	95	20	45	135	173	167	18.7
11	Right	77.8	5.87	5.45	174	100	20	45	167	175	171	19.0
12	Centre	72.1	7.05	6.01	163	95	25	45	141	146	154	20.7
								Mean	308	290	281	
								Loss%	-	6	9	

* Speed of P.M. drier too high.

fresh grass at one time at different drying temperatures. The fresh grass was weighed into wire-mesh baskets and kept in the oven until a constant weight was reached. However, it was desirable that any results should be capable of interpretation in terms of the Petrie-McNaught machine, and before proceeding with the investigation a comparison of the effect on the carotene content of the grass during drying in the commercial and laboratory machines was made.

(i) Comparison of commercial and laboratory grass driers.

A large sample of grass was taken from the load being fed to the Petrie-McNaught machine during a normal drying run and, after thorough mixing, divided into three parts. On one part the moisture, crude protein and carotene contents were immediately determined. A second portion was adequately marked for identification after drying and placed in the usual manner on the feed end of the Petri-McNaught drier. The third portion was dried in the laboratory machine under conditions of temperature and time of drying which had earlier been found to be efficient and reasonable. The dry grass from both machines was ground to a fine powder in a Christy and Norris hammer mill and analysed for moisture, carotene and protein contents. Samples were placed on the Petrie-McNaught drier at three positions across the width of the bed to give as representative results as possible. The experiment was carried out on two days when grasses of widely different carotene content were being dried. Table 20 records

the results; as usual, the figures for chemical determinations are the mean of duplicates.

From approximate calculations, the volume of drying air passing through each pound of fresh grass in the Petrie-McNaught machine was about 30-50 cu.ft./min. and in the laboratory drier about 80-90 cu.ft./min. These figures should be taken more as an indication of relative volumes rather than accurate data for each machine.

Considering the ever present difficulty of adequate sampling, the figures in Table 20 are reasonably consistent and indicate that drying by the Petrie-McNaught machine caused an average loss of about 6% carotene compared with the fresh grass and that in the laboratory drier the loss was about 9%. The original level of carotene in the fresh grass did not appear to affect this relationship. By the methods used the accuracy of carotene analyses is usually taken in this laboratory as ± 4% (mainly due to sampling error), hence the difference in carotene loss on the two driers would not appear to be significant. It seemed justifiable, therefore, to use the more easily controlled laboratory drier in further experiments concerning the effect of drying conditions on carotene content.

(11) <u>The effect of time and temperature of grass drying on carotene content.</u>

1 kg. of young, first-year ryegrass (8-12" high)

Table 21.

Effect of time and temperature of drying on carotene content.

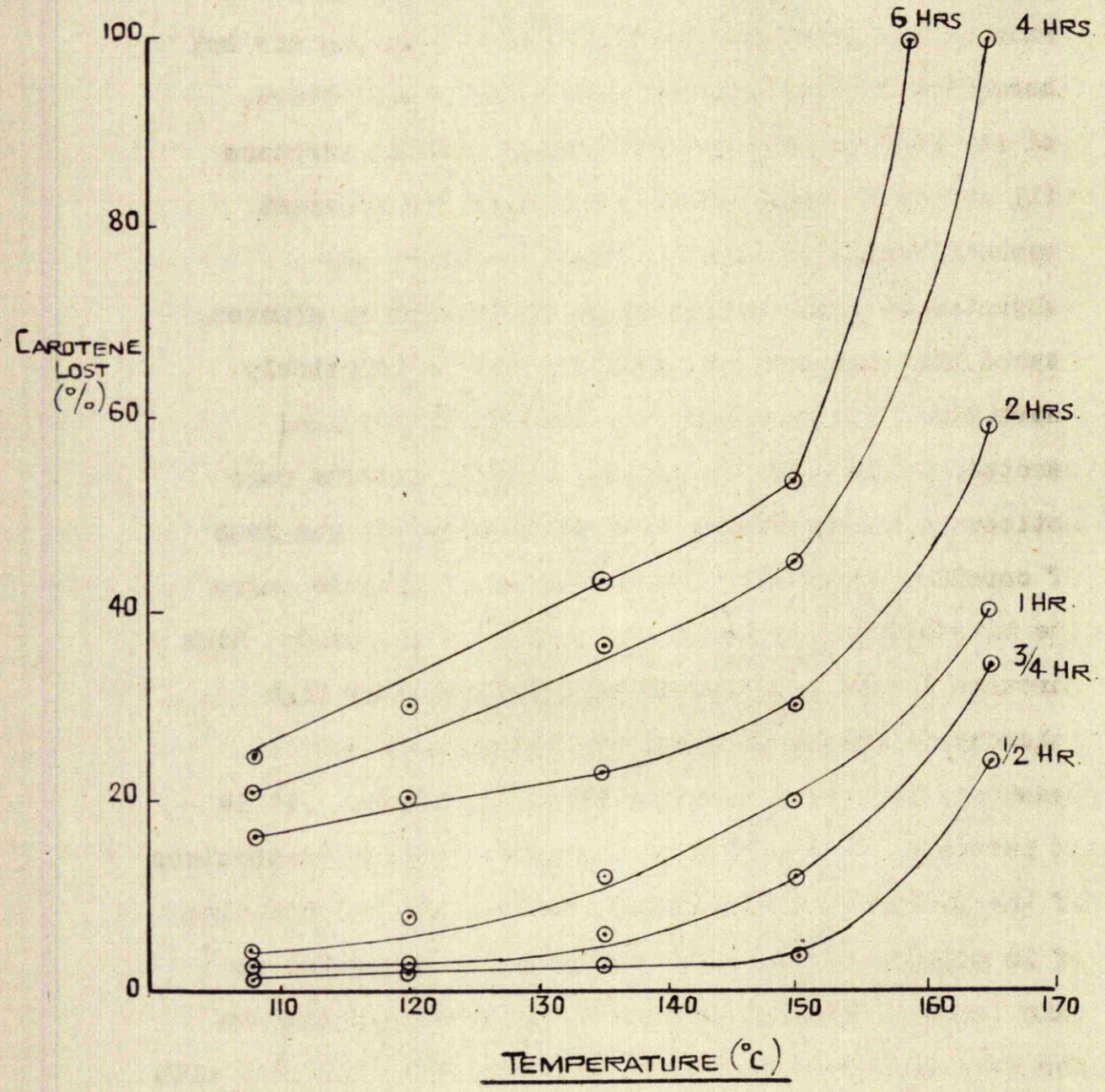
Temperature C.	Time of drying (Hr.)	Carotene (mg./kg. dry wt.) Fresh	Carotene (mg./kg. dry wt.) Dry	Carotene loss by drying (%)
104	¾	358	347	3
	1	358	341	5
	1½	358	316	12
	2	347	290	16
	4	347	281	19
	6	347	260	25
120	½	318	312	2
	1	318	297	7
	2	325	260	20
	4	325	212	35
	6	325	198	39
135	¾	367	357	3
	1	367	332	10
	1½	367	328	11
	2	389	286	26
	4	389	258	34
	6	389	209	45
150	½	366	355	3
	1	366	300	18
	2	370	278	25
	4	370	205	45
	6	370	175	53
165	1/3	223	188	16
	¾	223	142	36
	2	206	87	58
	4	206	nil	100
	6	223	nil	100

was cut, thoroughly mixed and divided into four portions. One portion was used immediately for carotene and protein analysis and the other three for drying under one set of conditions. For the next set of drying conditions a new sample of grass from the same field was cut and used similarly. In this way no sample of fresh grass was cut more than about half an hour at the most before it was dried, during which time it was kept in the dark at a low temperature. The fresh grass was dried in batches of 120 g. and at the end of the drying period was ground in the Christy and Norris hammer mill, collected in a brown bottle and analysed for carotene within 1-2 hours. Temperatures of 100 to 165°C. were used, with drying times ranging from 20 min. to 6 hours. No attempt was made to vary the drying air volume, which was between 80-100 cu.ft./min./1 lb. of fresh grass. The results are shown in Table 21. The carotene value at each temperature was plotted against time of drying, and from these curves data was obtained for the construction of Fig. 14 showing the loss of carotene in a fixed time of drying at temperatures between 100 and 170°C. (212 and 340°F.).

The smooth curves in Fig. 14 show, as might be expected, that lengthening the time of drying causes a considerable decrease in carotene content in the dried grass. It was previously known that at a drying temperature of 105°C. the time required to dry grass of 75-85% moisture content was 35-45 minutes,

FIG 14

EFFECT OF TIME & TEMPERATURE OF DRYING ON CAROTENE CONTENT OF FRESH GRASS.



consequently at higher temperatures it can be assumed that after some period of less than half-an-hour the grass was no longer losing moisture but was in fact being subjected to dry heat. It is probable that while grass is losing moisture the temperature of the grass is sensibly less than that of the drying air but thereafter it will quickly assume that temperature, and any heat sensitive constituents such as carotene will suffer. This possibly explains the apparent insensitiveness of carotene when the grass was subjected to temperatures up to 150°C. for 30 minutes. Beyond that temperature, even for such a relatively short time, the loss was over 20% of the original carotene content of the grass. Similar effects were noticeable as the drying time was increased, the loss of carotene increasing rapidly under conditions where the moisture in the grass was quickly evaporated. High carotene losses appeared to be paralleled by high chlorophyll losses although the latter were not measured, the grass becoming brown in colour. It is of interest to note that at the normal drying temperature of the Petri-McNaught machine, 540°F. (170°C.) the time of 16 minutes during which the grass is subjected to this temperature would appear to be about the maximum for safe drying. This is substantiated by the ease with which brown grass is produced if the temperature or speed of the belt are wrongly adjusted.

It should be emphasised that the data given in Tables 20 and 21 were obtained starting with fresh grass of 80-85% moisture content. Any pre-drying, either in the field or by the design of the drying machine would reduce the time required to cause carotene losses. Under good conditions of drier management it may be expected therefore that a carotene loss of from 5 to 10% of the amount present in the fresh grass will occur.

(c) Carotene losses during the storage of dried grass.

A commercial disadvantage of dried grass is the loss of carotene during storage. This is brought about by atmospheric oxidation and can be considerably influenced also by heat and light; the following experiments were designed to investigate these factors.

(i) The effect of sunlight on dried grass.

In this country, after drying, much dried grass is ground to a fine powder and stored in three-ply brown paper bags, a smaller amount is baled and stored without further protection from light. Two short experiments were carried out to discover the possible extent of carotene loss under both these conditions.

In the first, dried grass-meal in 0.8 g. lots was spread in a thin layer between two circular glass plates, 6.5 cm. diameter, and covered with one, two and three thicknesses of the same brown paper as used commercially. The plates were pressed tightly together to exclude as much air as possible and

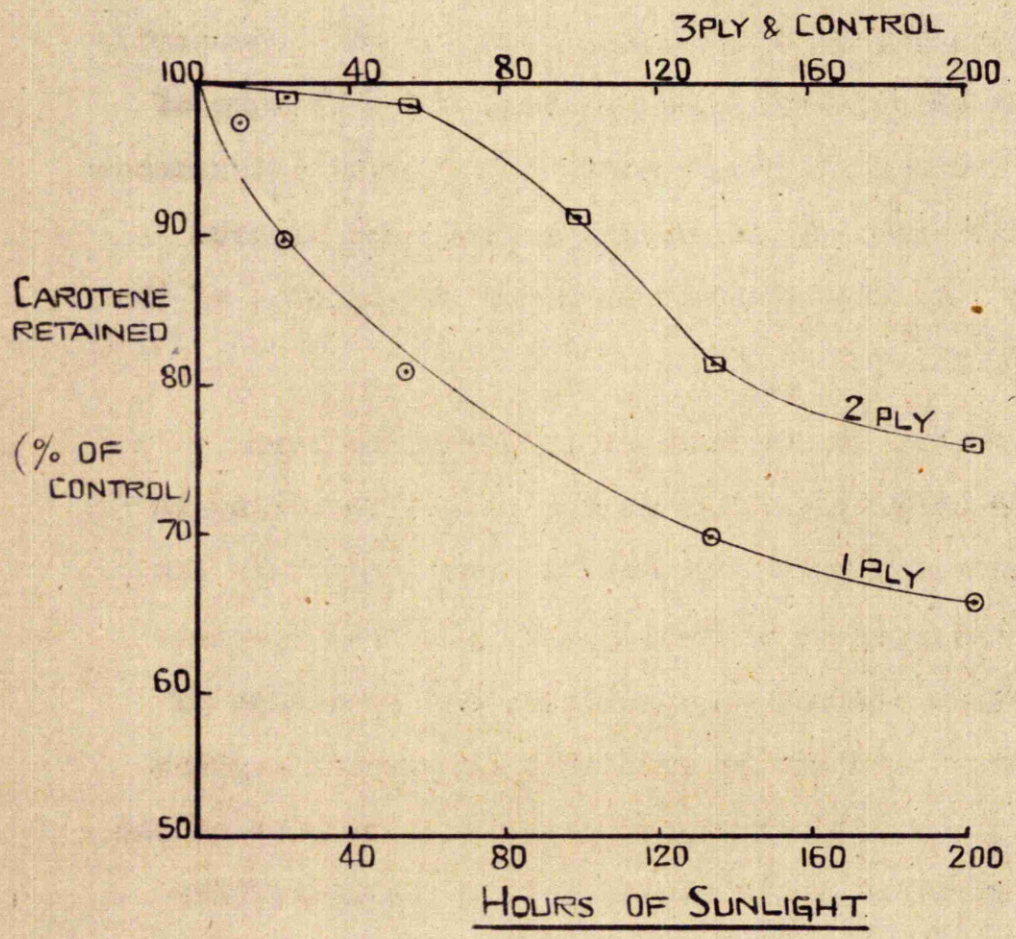
Table 22.

The protective power of brown paper in direct sunlight.

Hours exposed	Carotene content (mg./kg. dry weight)			
	1 ply	2 ply	3 ply	Control
0	342	342	342	342
12	336	-	-	-
24	303	-	-	338
55	266	329	-	332
135	235	247	298	304
202	227	207	267	268

FIG 15

EFFECT OF SUNLIGHT ON THE CAROTENE CONTENT OF DRIED GRASS COVERED BY BROWN PAPER



bound together at the edge with plasticene to exclude moisture and air. They were then placed outside on an open site and exposed to direct sunlight* for varying periods of time. It was realised that whatever the effect of the sunlight, in the warm weather prevailing there would be a certain amount of oxidative change of carotene from the air necessarily present between the glass plates. As a measure of this, control plates covered with several thicknesses of black photographic wrapping paper was exposed at the same time. Table 22 gives the results of the experiment.

In fig. 15 the loss of carotene has been plotted as a percentage of the amount remaining in the control sample. It will be seen that only the three thicknesses of brown paper afforded complete protection against the photochemical breakdown of carotene. It may be said that the use of a glass cover plate over the paper excluded some part of the solar spectrum which might have produced further carotene loss and this cannot yet be refuted. It has been noticed, however, that the dried grass in transparent glass bottles which have been exposed to sunlight through a glass window is quickly bleached of all pigmentation so that the retention of carotene by the samples was undoubtedly due chiefly to the

* exceptional August 1947 weather provided adequate sunshine.

Table 23.

The penetration of dried grass by sunlight.

Time in sunshine (hours)	Carotene (mg./kg. dry wt.)						
	Top	2nd	3rd	Layer 4th	5th	6th	Control
11	301	312	317	331	331	354	340
25	272	276	315	316	319	328	308
55	185	233	284	311	324	320	319
125	164	246	275	304	322	326	321

paper covers. Hauge & Aitkenhead (24) and Guilbert (20) have each concluded that ultraviolet light plays little part in the destruction of carotene. In the second of these two experiments on the effect of light, the degree of penetration of sunlight through dried grass was investigated by preparing nests of seven circular glass plates, such as used previously. A thin layer of dried grass meal sufficient just to cover the area completely was placed between each pair of plates. The seven plates were pressed tightly together, bound round the edges with plasticene and exposed to sunlight as before. Control plates similarly treated and wrapped in opaque black paper were exposed at the same time. At intervals a nest of plates was removed and the carotene content of the dried grass in each layer determined. The results are given in Table 23.

Each layer was approximately 0.3 mm. thick so that after 125 hours of direct summer sunshine the carotene content of grass only to a depth of approximately one millimeter had been affected and that only to an extent of 6%.

From these two experiments it would appear that brown paper bags of triple thickness will adequately prevent carotene losses due to light and that where dried grass is baled without grinding, a thin surface layer is likely to lose considerable carotene but the major bulk of the material should suffer little damage from this source.

Table 24.

Initial data of baled dried grass.

Bale No.	15	17	18	21	22
(%) moisture	8.6	9.6	8.0	10.6	8.5
(%) crude protein	10.8	10.9	10.5	10.0	8.6
carotene (mg./kg. dry wt.)	111	122	112	122	93

An opportunity to follow carotene changes in baled dried grass occurred during the course of this work, but unfortunately not under conditions which would allow the additional effect of exposure to light to be isolated from oxidative losses. The grass had been wilted in the field for 6 hours prior to drying on a Petrie-McNaught band drier. It was stacked, left in the open overnight and baled the next day; treatment which had caused an appreciable and non-uniform rise in the moisture content. The bales, measuring 3½' x 2½' x 1½' weighed approximately 100-110 lb. and varied in density from a tightly packed mass along the "bottom" to loosely held grass along each side. To allow, to some extent, for non-uniformity of material, each bale was immediately sampled at 10-15 points over its surface (using a wide cork borer), the samples bulked, ground and determinations of moisture, carotene and crude protein made. The corresponding data for the stored bales were then compared with these figures rather than with an initial average of all the bales. Table 24 shows that the variation between bales was not more than might be expected in different samples from a field of grass; the higher moisture content of some might have been caused because they contained more grass from the outside of the stack of bales.

The bales were stacked in a Dutch barn with the "bottoms" of all the bales protected from the light

by the manner of stacking, but with sides and ends of some exposed and, in the case of the top bales, the top surfaces also. One bale was taken from the stack at fortnightly intervals, a sample picked from each surface which had been exposed to light and the bale then opened. A section, about 5 inches thick was lifted in one piece from about the middle of the bale and eight samples taken at points indicated in the diagram.

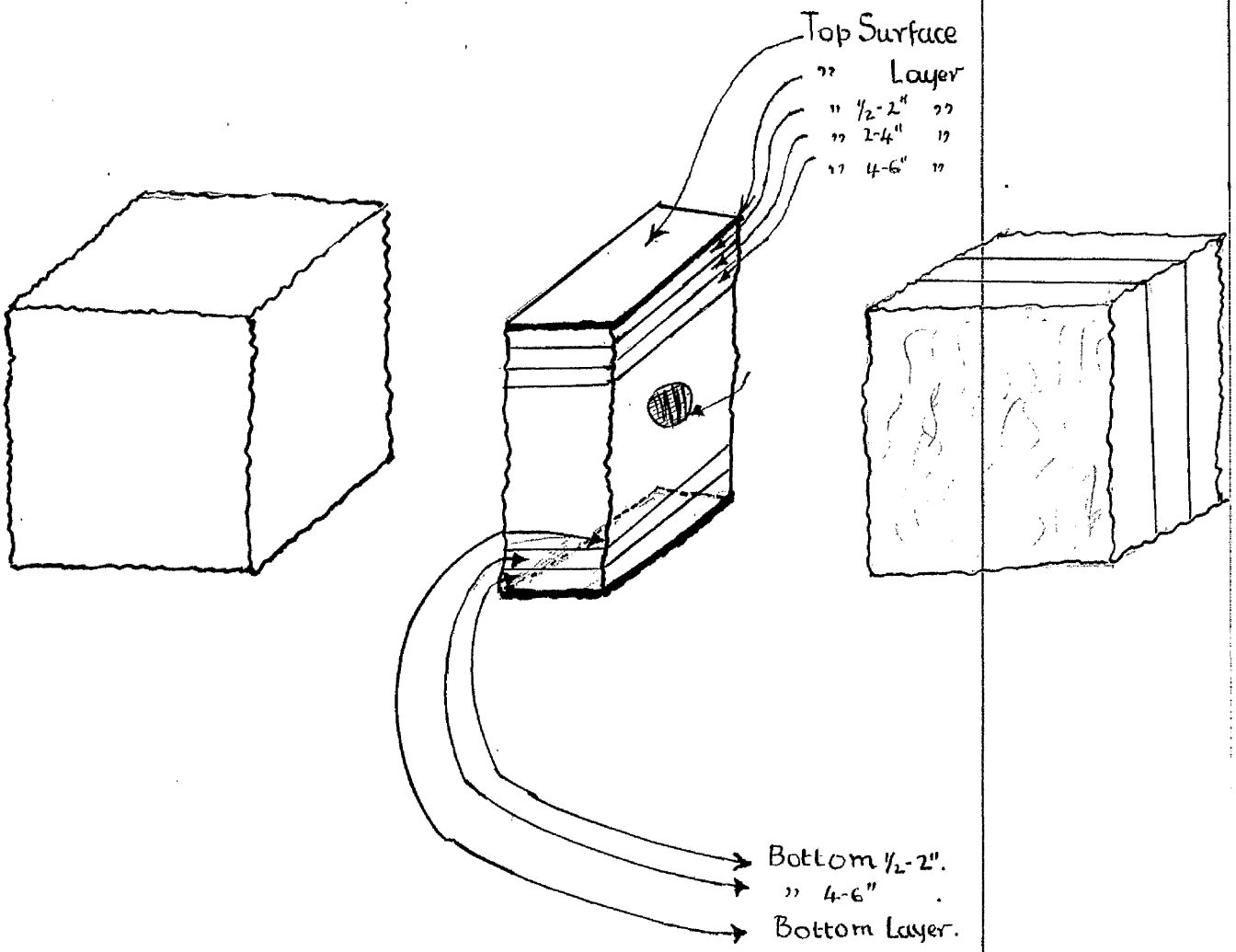


Table 25.

Changes occurring during storage of baled, dried grass.

C = %age carotene retained, P = crude protein (%), M = moisture (%)

Bale No.	22			21			17			18			15		
Period stored (weeks)	2			4			6			8			10		
	C	P	M	C	P	M	C	P	M	C	P	M	C	P	M
Top surface	-	-	-	-	-	-	26	10.9	10.5	6	9.8	11.4	4	10.4	13.1
" layer	90	10.9	8.9	70	10.3	13.1	70	10.0	11.0	59	10.0	11.4	44	10.5	11.9
" ½" - 2"	100	9.4	9.1	72	9.0	12.8	74	10.3	11.9	60	10.2	11.1	70	10.2	13.7
" 2" - 4"	100	8.5	8.4	76	9.6	12.7	83	9.7	11.5	65	9.9	10.9	72	10.0	12.3
" 4" - 6"	100	9.3	7.9	85	10.2	12.1	89	11.0	10.8	71	9.8	10.3	75	10.5	12.2
Centre	100	10.5	8.0	85	9.8	11.5	88	10.1	12.1	87	10.4	10.8	75	10.5	11.8
Bottom 4" - 2"	100	10.5	8.2	85	9.5	11.7	94	9.9	11.0	85	9.8	10.8	78	10.4	12.6
" 2" - ½"	100	8.6	9.1	91	10.1	12.1	92	9.1	11.4	91	10.3	11.4	80	9.5	12.5
" layer	100	10.2	8.3	84	9.8	12.6	94	10.1	11.5	91	10.6	11.4	85	10.3	12.8
Average (excluding top surface)	99	9.7	8.5	81	9.8	12.3	85	10.0	11.3	76	10.1	11.1	72	10.2	12.5
Analyses at start	100	8.6	8.3	100	10.0	10.6	100	10.8	9.6	100	10.5	8.0	100	10.8	8.6

In general, the colour disappeared remarkably quickly from the surfaces exposed to the light; after only two weeks some of the surface grass was almost bleached of green colour. The samples designated "Top surface" in Table 25 were from such grass, taken to indicate the maximum loss of colour, the sample "Top layer" included some of the top surface but extended to a depth of about $\frac{1}{2}$", so that the effect of the bleached top layer was not predominant. The carotene contents in Table 25 have been expressed not in quantitative terms but as a percentage relative to the carotene content of the bale when first formed. It has already been shown that, in agreement with other workers, a high correlation between carotene and protein exists in fresh grass and although this relationship may be affected by wilting and drying, protein content can still be a useful comparative guide. Hence protein values are given for each sample taken from the stored bales. The average of the values for all layers except the top surface is included and although this is certainly not a weighted mean, it probably summarises quite well the general trend.

The great effect of exposure to light on carotene is shown in the top surface samples and to a lesser extent in the top $\frac{1}{2}$" layer. The bottom layers were virtually protected from light and any carotene change in them can be ascribed almost entirely to oxidative

change. It has been suggested above that once the thin covering layer of a bale is bleached the penetrative effect of sunlight on carotene should be negligible, yet as Table 25 shows, in all bales there is a steady decrease of carotene from bottom to top. The apparent contradiction is probably explained by the varying density in the bale, which is greatest at the bottom and least at the top and sides. Passing from the bottom to the top surface of the bale the amount of available oxygen increases, and over short periods of storage, will almost certainly affect the rate of carotene oxidation.

The moisture content had increased appreciably in 10 weeks (May-August) and may be expected to rise further in the winter months. The protein contents of the various layers in different bales are remarkably uniform. It is too early to say whether the slight increase with storage time is real or accidental. Loss of dry matter in stored feedingstuffs has been reported by Snow & Wright (47), causing an apparent rise in the percentage of the non-decomposing constituents and this may be manifested more clearly when the bales have been stored for a longer time.

(ii) <u>The influence of available oxygen on carotene losses.</u>

In the control samples mentioned already considerable loss of carotene occurred which could only be ascribed to oxidation by the air surrounding

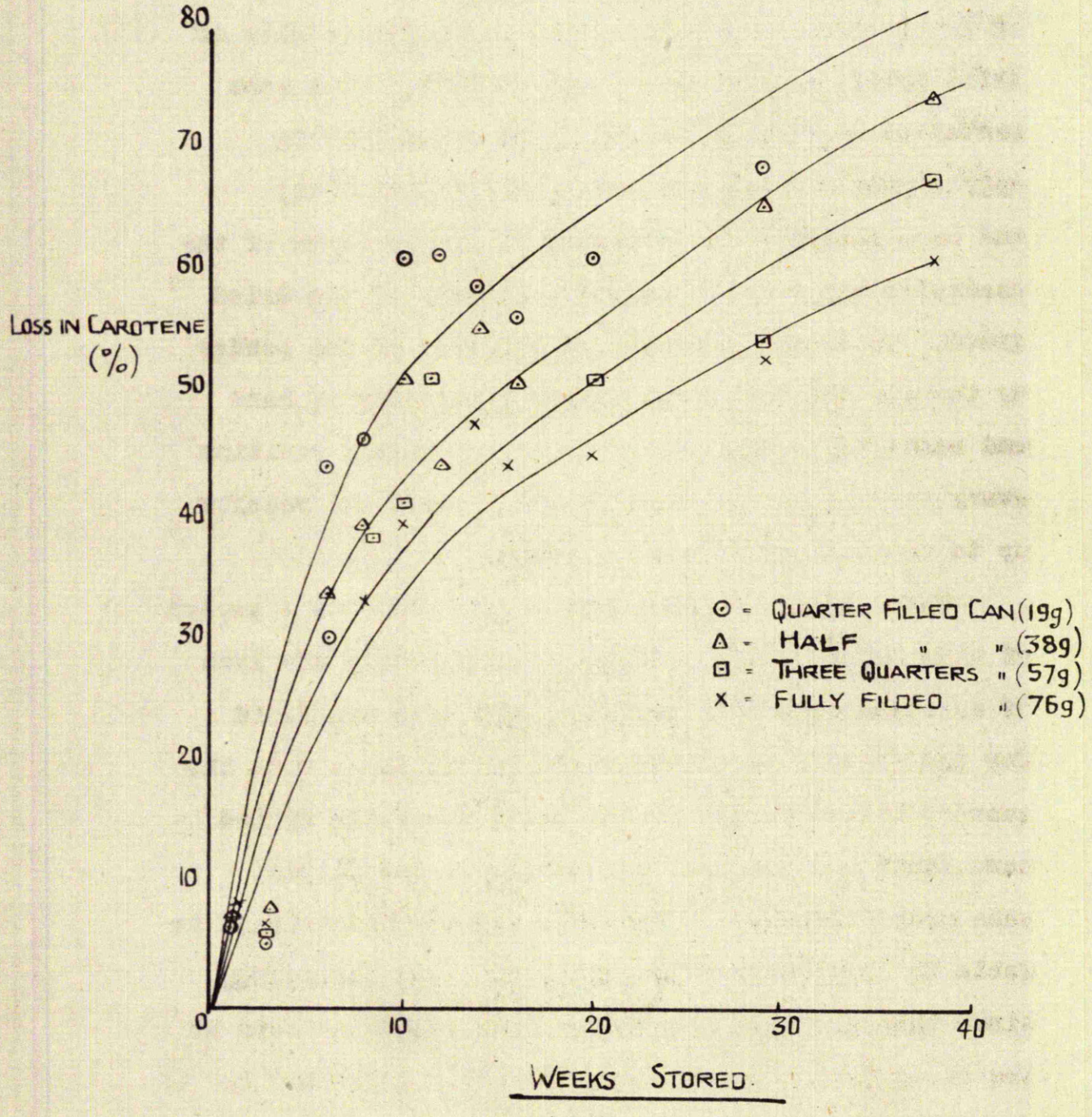
Table 26.

Grass meal stored at 25°C. with varying amounts of air.

Period stored (weeks).	Quarter-filled (19 g.)			Half-filled (38 g.)			Three-quarter filled (57 g.)			Fully-filled (76 g.)		
	O_2 (%)	CO_2 (%)	Carotene (mg./kg. dry wt.)	O_2 (%)	CO_2 (%)	Carotene (mg./kg. dry wt.)	O_2 (%)	CO_2 (%)	Carotene (mg./kg. dry wt.)	O_2 (%)	CO_2 (%)	Carotene (mg./kg. dry wt.)
0	20.85	0.05	393	20.85	0.05	393	20.85	0.05	393	20.85	0.05	393
1	20.09	0.06	367	19.57	0.07	363	19.15	0.05	370	18.71	nil	360
3	18.84	0.49	375	18.14	0.87	362	16.94	0.89	368	16.09	1.15	369
6	18.46	0.63	220	16.71	0.86	253	15.34	1.22	274	13.09	1.62	267
8	18.00	0.58	211	15.81	1.13	237	13.81	1.65	243	11.66	2.01	260
10	17.60	0.71	153	15.27	1.20	191	12.66	1.92	225	12.38	2.37	240
12	16.99	0.76	151	14.37	1.37	166	13.85	2.03	191	9.27	2.41	216
14	17.59	0.86	162	13.45	1.54	175	10.60	2.08	160	7.89	2.63	206
16	16.89	0.78	172	12.89	1.70	193	11.44	2.04	205	8.17	2.97	227
20	16.51	1.02	152	12.88	1.70	174	9.13	2.57	191	5.73	2.80	217
29	15.46	1.11	123	10.18	2.23	136	6.54	3.24	173	2.93	4.15	187
38	15.35	1.24	72	9.91	2.42	93	5.89	3.35	124	2.35	4.29	152

FIG 16

EFFECT OF AVAILABLE OXYGEN ON CAROTENE CONTENT OF DRIED GRASS STORED AT 25°C.

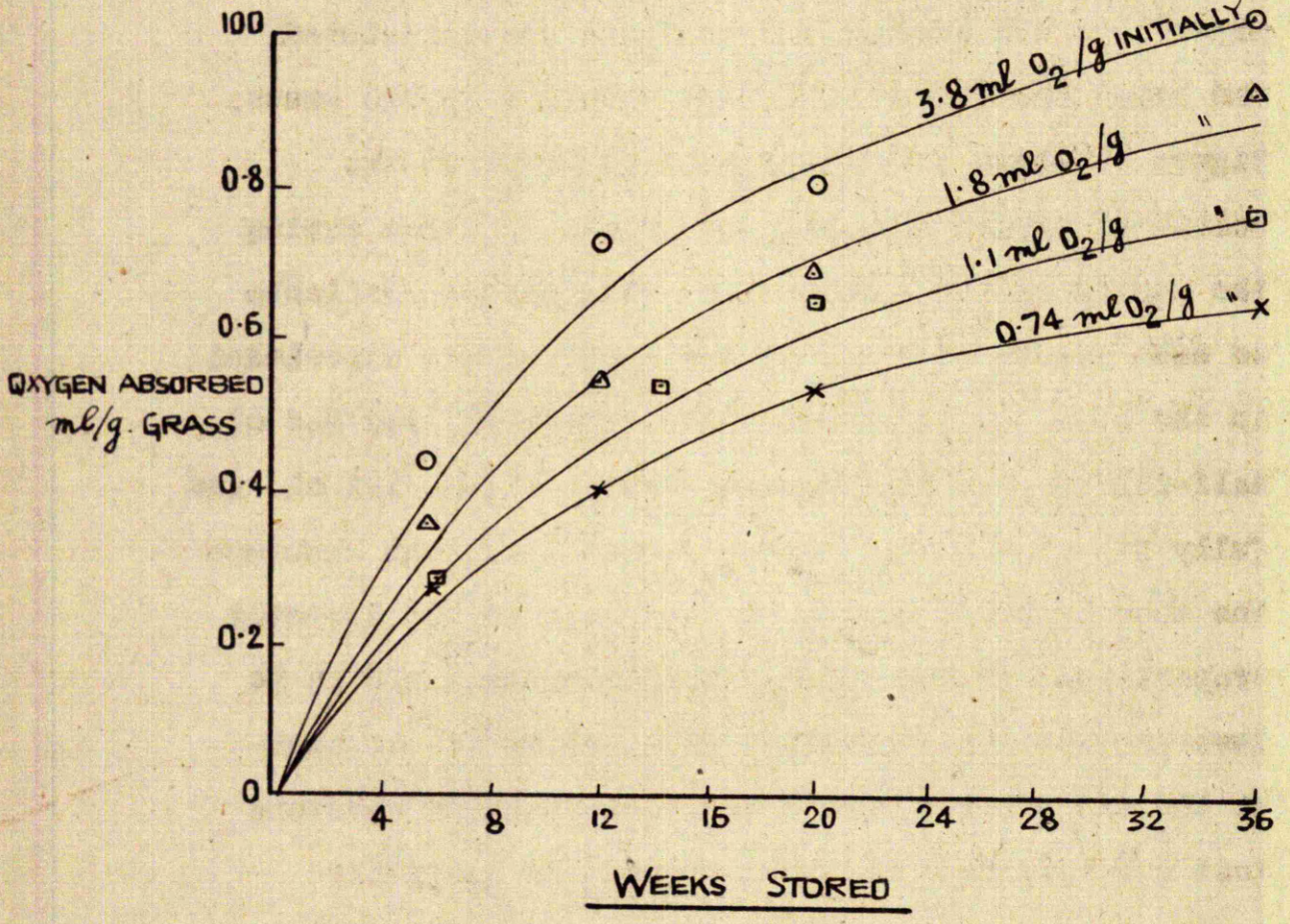


the particles of dried grass and in the stored bales more carotene was lost in the looser, top layers. In order to investigate whether oxygen availability was a major factor in the amount of carotene lost, a number of 12 oz. cans were filled with different weights of dried grass, closed and stored at 25°C. The cans contained 19, 58, 57 and 86 g. of grass (giving approximately $\frac{1}{4}, \frac{1}{2}, \frac{3}{4}$ and completely filled cans) and were analysed at intervals for composition of the headspace air and for carotene content of the dried grass. To ensure adequate penetration of the powder by the air the cans were shaken vigorously by hand and returned to the incubator in a reversed position every two days. Figures 16 and 17 show the results up to the end of 36 weeks storage.

Table 26 gives the results obtained over a period of nine months. Fig. 16 shows graphically the loss of carotene over this period. The loss was rapid for the first nine to ten weeks in all cans, with the quarter filled series losing most, the fully filled cans least and the half and three-quarter filled cans proportionally. The data for gas composition in Table 26 indicates only partly what was occurring, since the amount of air in the four series of cans at the commencement of the experiment was different in each series. In order to know the true volume of air it was necessary to measure the density of the grass at 25°C. Normal liquids used in pyknometry cannot be used because of their tendency to extract

FIG. 17

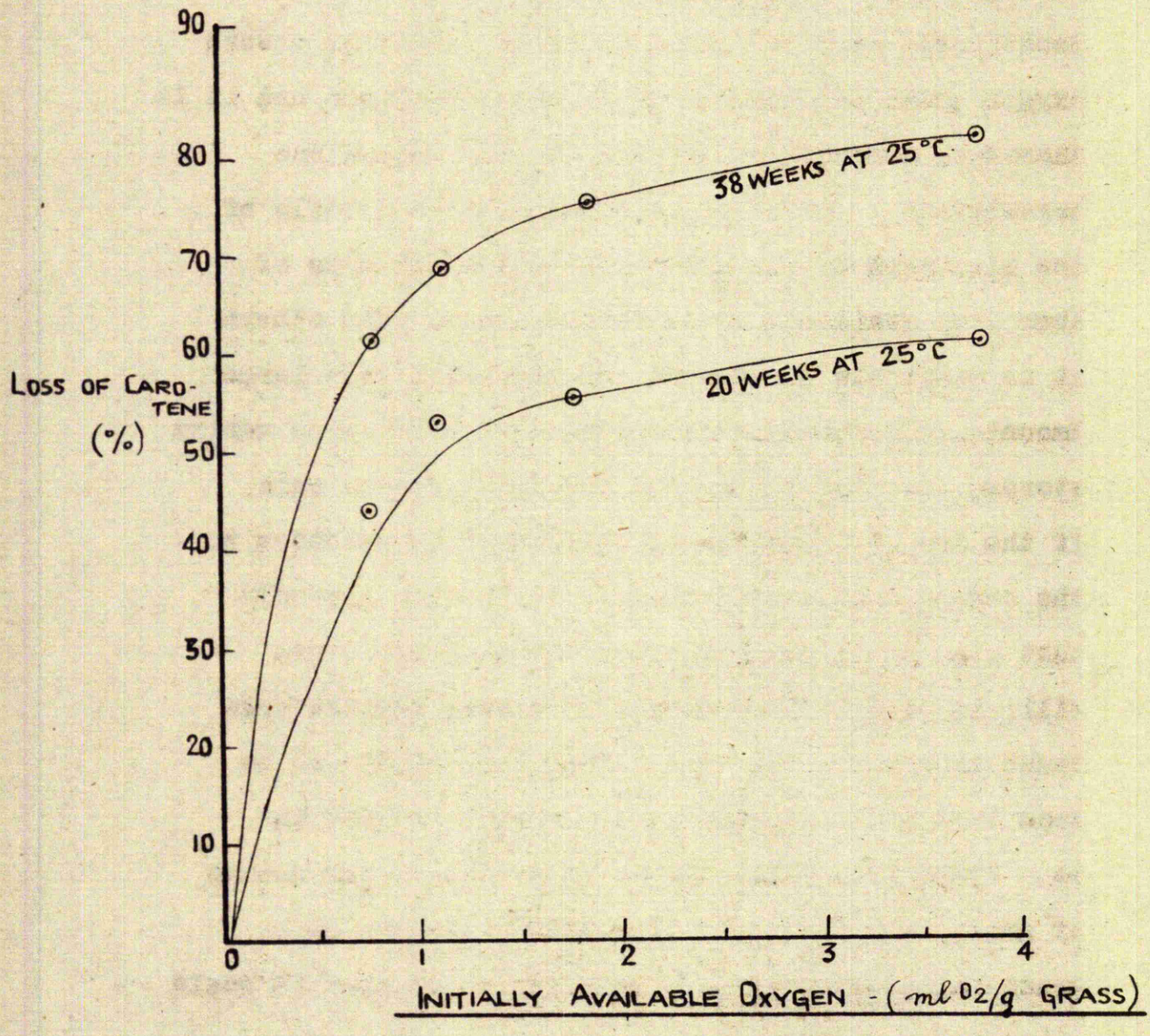
CONSUMPTION OF OXYGEN BY DRIED GRASS DURING STORAGE AT 25°C.



some constituent from the grass. After several trials it was found that liquid paraffin could be used without any appreciable extraction occurring. The density of this sample of grass at 25°C. was 0.80. From a knowledge of the total volume of the closed cans and the percentage of oxygen in the air in the can, the volume of oxygen at the start of the experiment and at each gas analysis was calculated and hence the volume of oxygen absorbed by the grass. Figure 17 shows this absorption in terms of the volume of oxygen absorbed per gramme of grass during the period of the experiment. The oxygen available to each gramme of grass at the start of the experiment in the four series of cans was, quarter-filled 5.8 ml., half-filled, 1.8 ml., three-quarter filled, 1.1 ml. and fully filled 0.8 ml. These curves show that although the absorption of oxygen by the grass is not directly proportional to the volume initially available it is nevertheless considerably influenced by it, so that at the end of nine months the percentage of carotene lost corresponding to these volumes of initially available oxygen are 80, 75, 70 and 61% respectively. At all times during the experiment the small amount of grass in the quarter-filled can was absorbing more oxygen from the air within the can than in any of the other series. Considering Figs. 16 and 17 the similarity in shape is striking and if loss of carotene is correlated with volume of oxygen absorbed

FIG. 18.

EFFECT OF AVAILABILITY OF OXYGEN ON CAROTENE LOSS.



by one gramme of grass, taking half a dozen points from the curves relating to each of the four fillings, it was seen that a straight line passing through the origin could be drawn about which such points are fairly evenly distributed. Carotene is only one constituent of dried grass which is likely to absorb oxygen under the conditions of the experiment and it is therefore somewhat surprising to find such close correlation. Unless some physiological details of the placement of the chromoplasts renders some of them less available to oxidative attack than others it is difficult to account for the relatively large amounts of carotene still unattacked after nine months storage at 25°C. in all but the fully filled cans. If the loss of carotene is considered in relation to the oxygen initially available it becomes apparent that even small quantities of air in a container will, in five to nine months at normal temperatures cause appreciable losses. From Fig. 18 it can be seen that at 25°C., 10% carotene will be lost in 10 to 12 weeks if 0.1 ml. oxygen is available per gramme of grass, i.e. in terms of a can* filled by 40 g. grass, about 3% oxygen. This suggested that it would be interesting to reduce the oxygen content of the air surrounding grass meal to this extent and investigate the carotene losses resulting during storage.

* 6 oz. cream can.

Table 27.

Grass meal stored in air and nitrogen at different temperatures.

Period stored (weeks)	0°C AIR O_2 (%)	0°C AIR CO_2 (%)	0°C AIR Carotene (mg./kg. dry wt.)	0°C NITROGEN O_2 (%)	0°C NITROGEN CO_2 (%)	0°C NITROGEN Carotene (mg./kg. dry wt.)	20°C AIR O_2 (%)	20°C AIR CO_2 (%)	20°C AIR Carotene (mg./kg. dry wt.)	20°C NITROGEN O_2 (%)	20°C NITROGEN CO_2 (%)	20°C NITROGEN Carotene (mg./kg. dry wt.)
0	20.8	0.05	393	-	-	393	20.8	0.05	393	-	-	393
2	20.18	0.18	369	4.53	0.06	354	17.74	0.41	332	1.07	0.33	362
4	19.09	0.21	359	0.94	0.09	403	17.23	0.91	318	0.62	0.37	374
5	19.28	0.19	367	0.89	0.15	384	15.67	0.91	359	0.10	0.31	358
6	-	-	-	-	-	-	15.24	1.02	350	0.12	0.39	378
8	18.65	0.25	355	1.19	0.19	359	14.64	1.33	312	0.55	0.43	359
10	18.32	0.27	305	1.96	0.09	336	13.00	1.54	247	0.65	0.36	316
12	16.73	1.49	322	1.06	0.14	326	11.15	1.76	278	0.55	0.53	311
14	17.17	0.44	315	0.37	0.07	343	11.41	1.60	262	0.19	0.52	342
16	17.00	0.46	345	0.34	0.20		10.32	1.84	267	0.44	0.49	361
20	16.15	0.53	312	0.13	0.06	373	7.26	2.40	251	0.05	0.49	357
25	14.79	0.90	292	0.15	0.40	390	5.65	2.81	234	-	0.61	385
34	13.24	0.51	293	0.62	0.45	378	4.57	2.75	194	0.76	0.33	379
	37°C AIR			37°C NITROGEN			45°C AIR			45°C NITROGEN		
0	20.8	0.05	393	-	-	393	19.58	0.24	312	1.10	0.14	363
5 hr.							18.49	0.75	329	0.92	0.53	325
10 "							17.65	0.92	287	0.52	0.64	302
24 "							15.77	1.55	307	0.34	0.79	319
50½ "							13.10	2.40	292	0.13	0.39	300
98½ "							6.98	5.26	260	0.18	1.55	344
1 wk.							3.25	7.51	271	0.27	2.72	316
1½ "							4.39	6.50	181	0.51	2.37	293
2	12.24	0.07	323	1.26			1.44	7.91	214	0.75	3.08	355
3							1.53	8.75	214	1.25	3.04	352
4	7.99	4.31	305	0.55	1.37	395						
5	6.74	4.49	255	0.71	1.51	377	1.86	9.89	206	1.07	4.25	340
6	5.81	5.43	228	0.50	1.37	329						
7							nil	12.18	214	nil	6.15	375
8	4.56	6.00	229	0.87	1.86	359						
10	2.34	6.69	199	0.89	1.63	292						
12	1.89	6.79	177	0.37	2.68	306						
14	0.37	8.32	227	0.21	2.50	345						
15												
16	0.82	7.93	229	0.59	2.44	363	0.06	16.48	206	-	7.98	364
20	0.13	8.61	222	nil	2.74	370						
25	-	8.68	215	-	3.11	372						

The increase in carbon dioxide concentration will be discussed later (p.104).

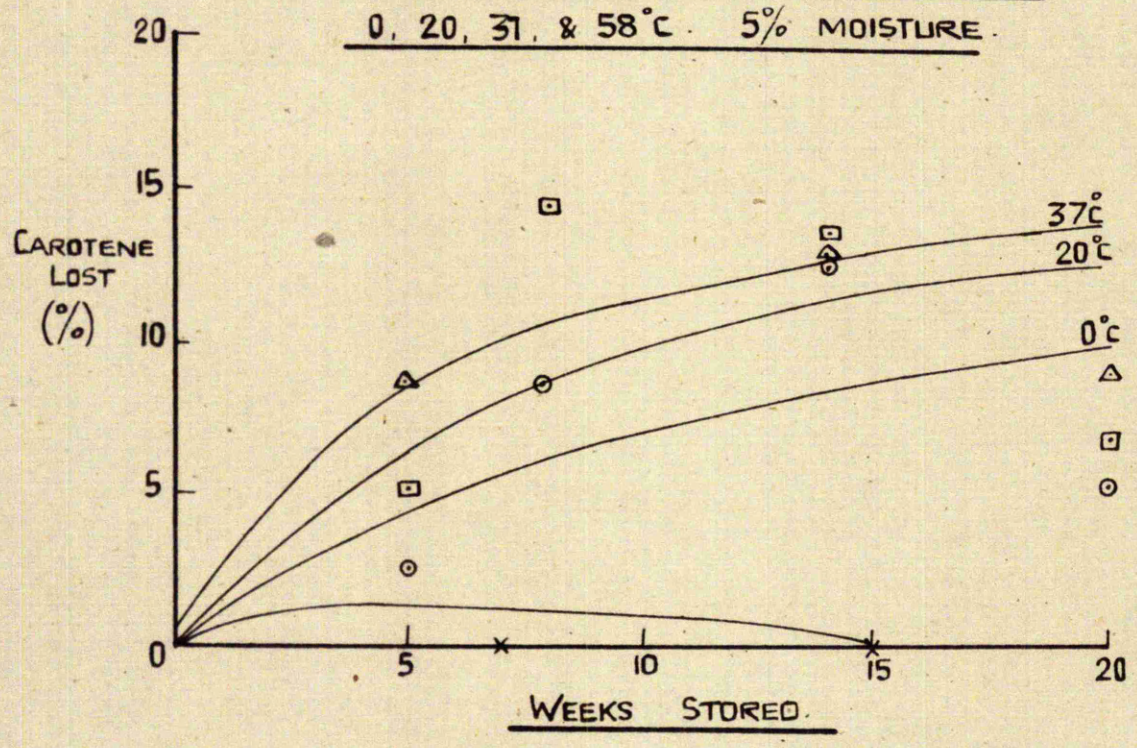
(iii) The effect of nitrogen packing on carotene losses in dried grass.

The technique used in gas packing full-cream milk powder, described by Lea, Moran & Smith (31) was used. Open top 6 oz. cream cans filled with dried grass powder were closed with the usual double seaming machine and a small hole pierced in one end. The cans were placed in a cabinet and evacuated at 30" Hg. for 5 minutes. The vacuum was then released with nitrogen to a positive pressure of 4 lb./sq.in. and the cycle repeated once more. The brogue holes were then quickly soldered and the cans stored. In this way the oxygen content was reduced to about 4%. It was decided to investigate at the same time the effect of temperature on carotene loss and cans were stored at 0, 20, 37 and 58°C. Cans which had not received the gas packing treatment were stored for comparison at the same time. Cans were removed at fortnightly intervals for the first four months and thereafter at longer intervals, for analysis of the gas composition within the can and for carotene content of the dried grass. Table 27 records these values; Figs. 19 and 20 show a comparison of carotene losses and oxygen absorption respectively in the nitrogen and air packed cans.

The preservative effect of the inert gas on the

FIG 19.

GRASS POWDER STORED IN NITROGEN AT 0, 20, 31, & 58°C. 5% MOISTURE.



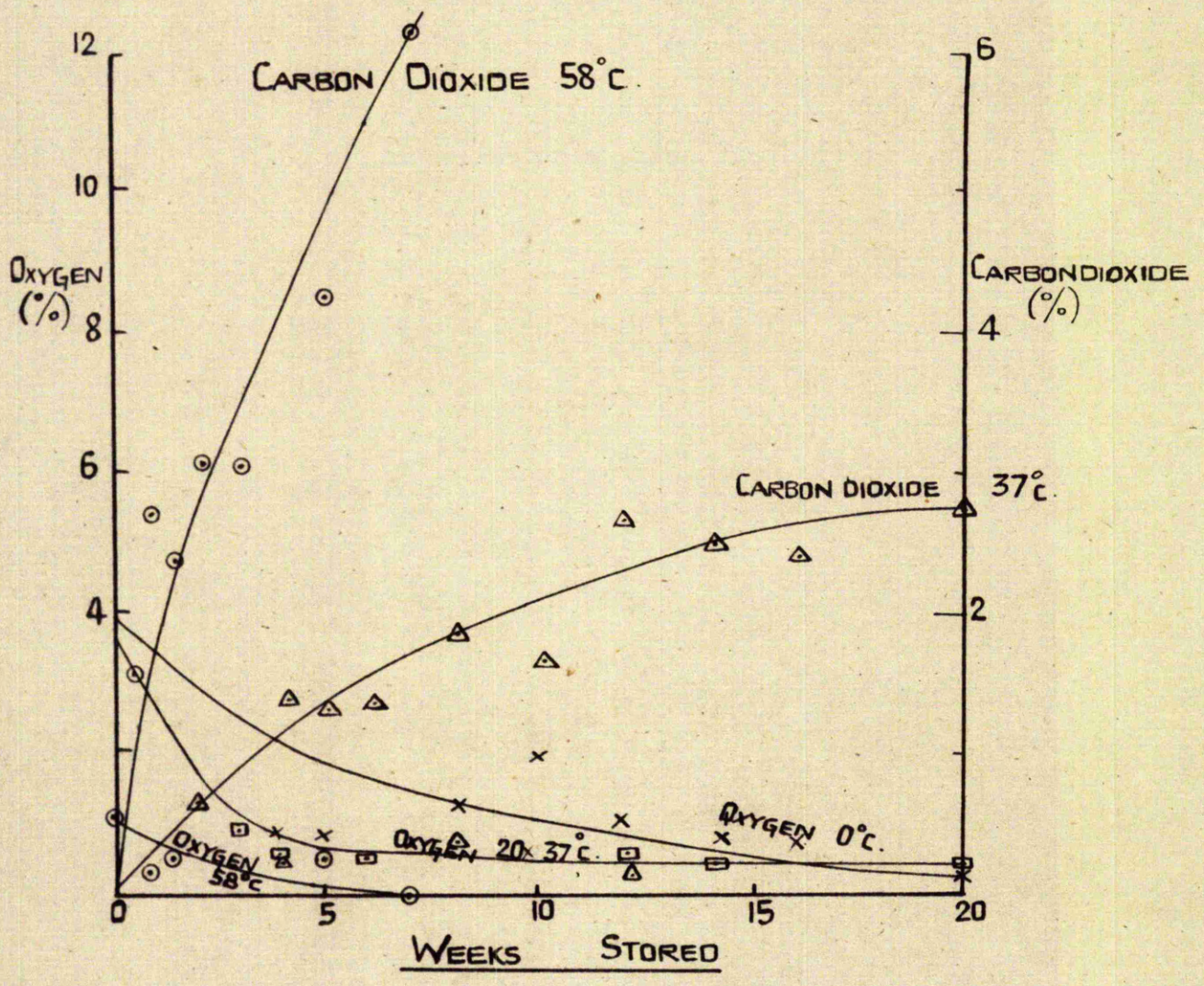
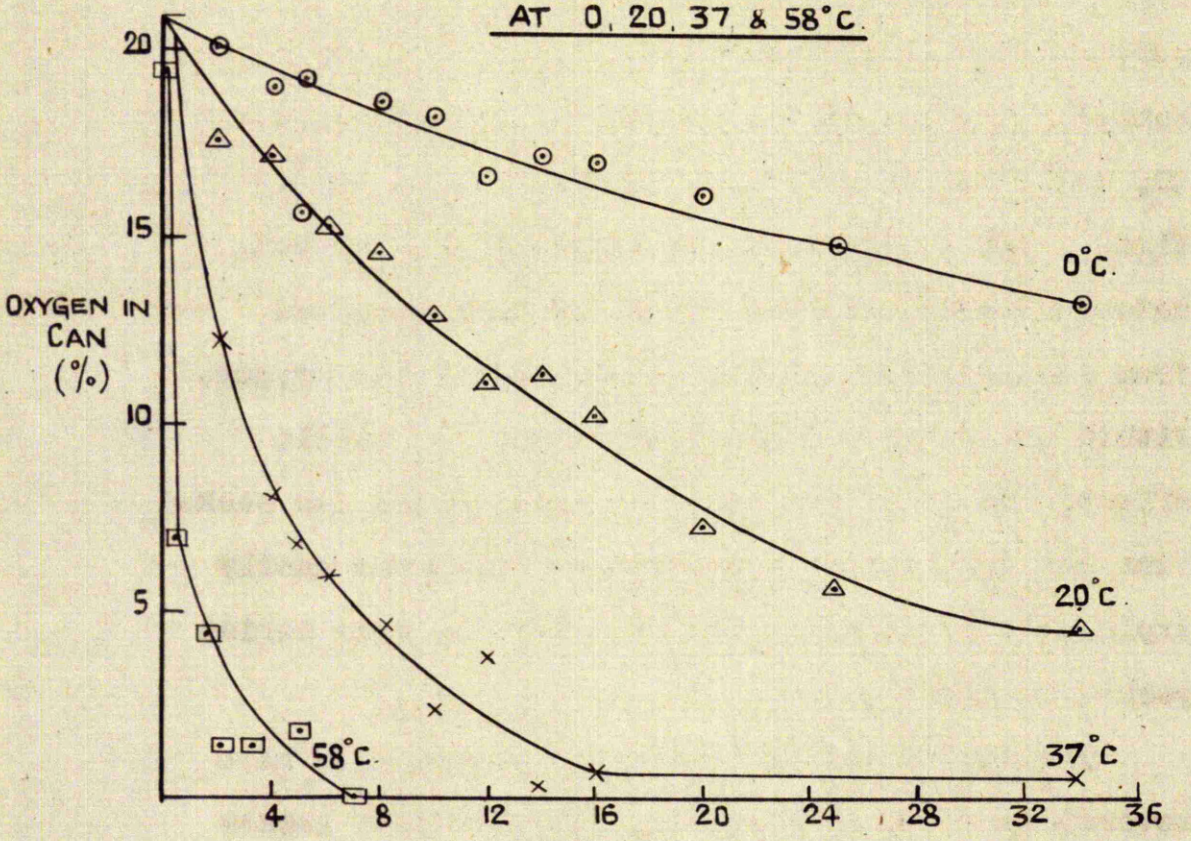
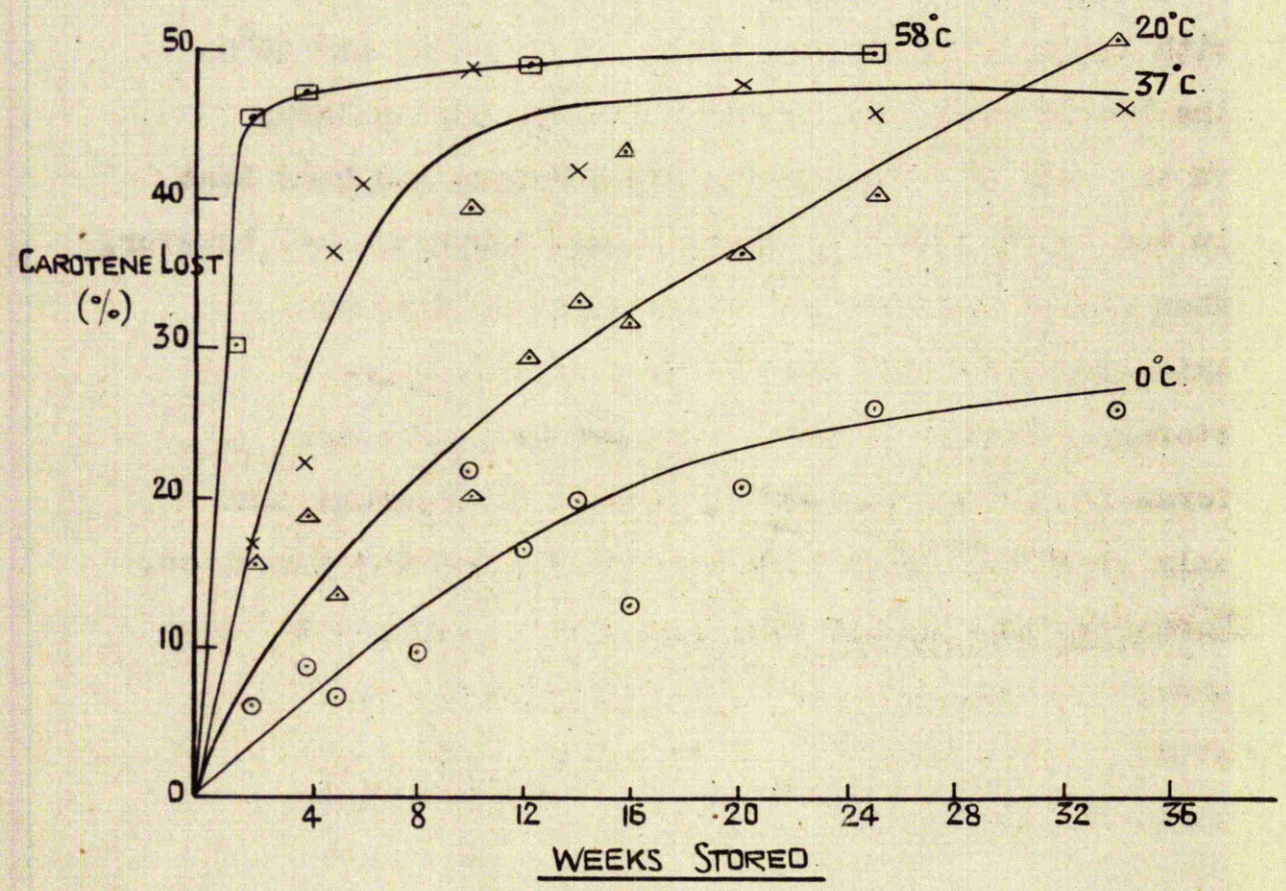


FIG. 20.

STORAGE OF DRIED GRASS IN AIR AT 0, 20, 37 & 58°C.





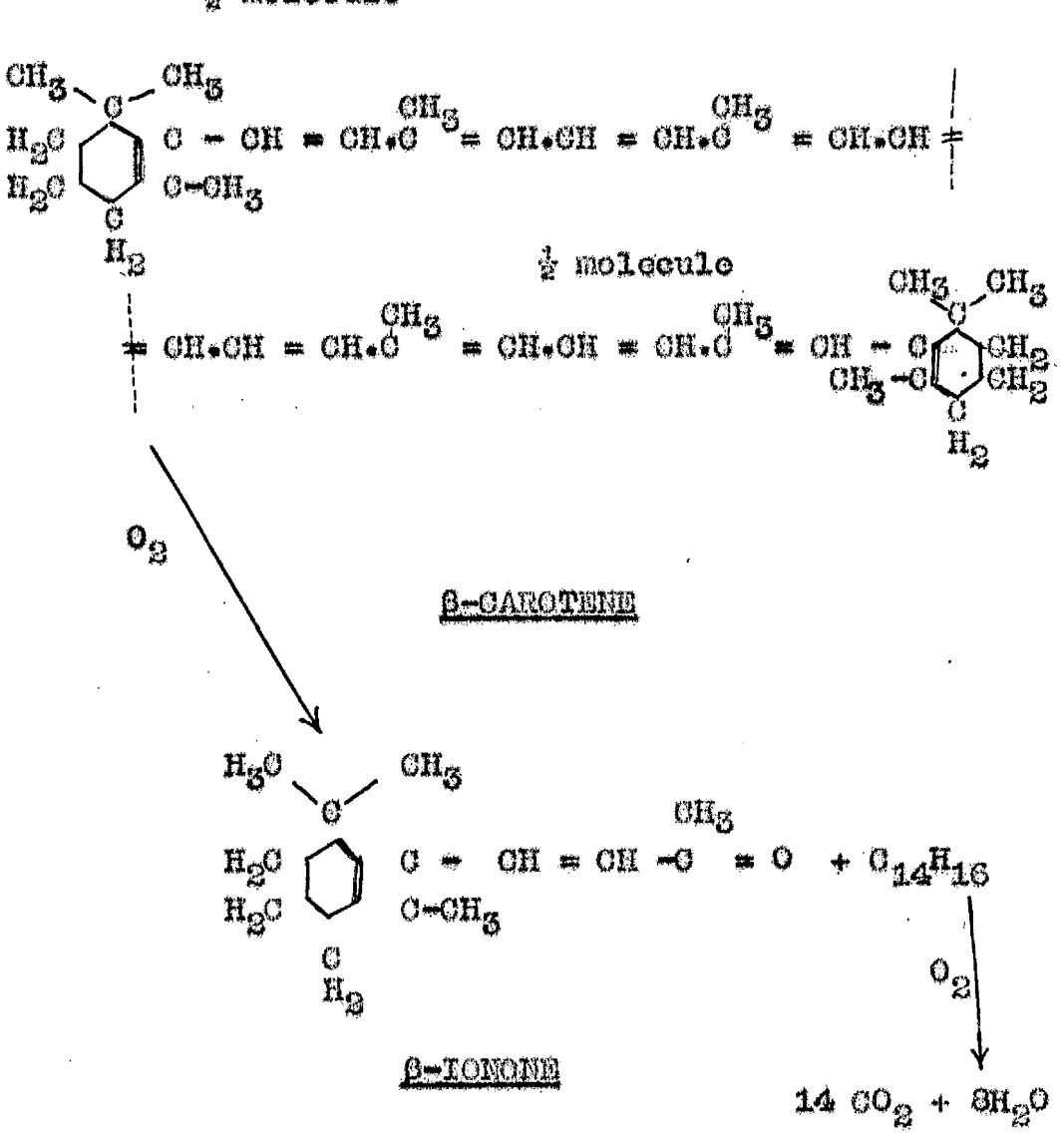
carotene is very marked at all temperatures but particularly so at the highest, 58°C. The carotene content of grass in the nitrogen packed cans at 0, 20, and 37°C. after slight initial losses during the first three months remained appreciably constant between 90-95, which is about the loss expected from consideration of the initial available oxygen. Within the range 0-37°C. temperature had little effect; at 58°C. the carotene loss in the gas packed cans was negligible, a surprising fact more easily explicable considering the data for the same series packed in air.

The data relating to the air packed cans have several interesting features. The carotene losses of grass meal stored at 0 and 20°C. slowly increased with lengthening storage time. At both 37 and 58°C. the losses were very rapid initially, particularly in the cans at 58°C., where 17% carotene had been lost in ten hours. At both these higher temperatures, however, when 40-45% carotene had disappeared no further oxidation occurred, even after a further 28 weeks storage. If the data relating to carbon dioxide formation is now considered it will be apparent that only at 37 and 58°C. were appreciable amounts generated. Moreover, at the time when the carotene losses in the grass at these two temperatures were firmly settled at 40% (12-14 weeks at 37°, 2 weeks at 58°C.) the carbon dioxide content in both series had risen to

about 8%. More important than the formation of carbon dioxide is the fall in oxygen content which had been, at these times, reduced to about 2% at both temperatures. This again confirms the results already reported which showed that if the available oxygen is reduced by any means to a figure of the order of 0.1 ml. per gramme of grass the loss of carotene will be small, at most not more than 10%. In the air-packed cans this reduction was obtained partly at the expense of the carotene but as soon as it had been achieved no more carotene was oxidised. As Mitchell, Schenk & King (56) accidentally showed, if the container is opened from time to time so that the carbon dioxide is lost and fresh air introduced, the carotene losses are progressive. This explains why the wide range of storage temperatures in the gas-packed series had little effect, because after a very short time the available oxygen had fallen below 0.1 ml./g. and in the absence of sufficient oxygen the carotene was stable over the range 0 - 58°C.

It may be apposite at this point to return briefly to the series of cans of grass containing different amounts of air. From Table 26 it will be seen that only in the fully filled cans had formation of carbon dioxide risen high enough to lower the available oxygen content to this (arbitrarily chosen) figure of 0.1 ml./g. grass, consequently at the end of nine months the carotene losses were still rising.

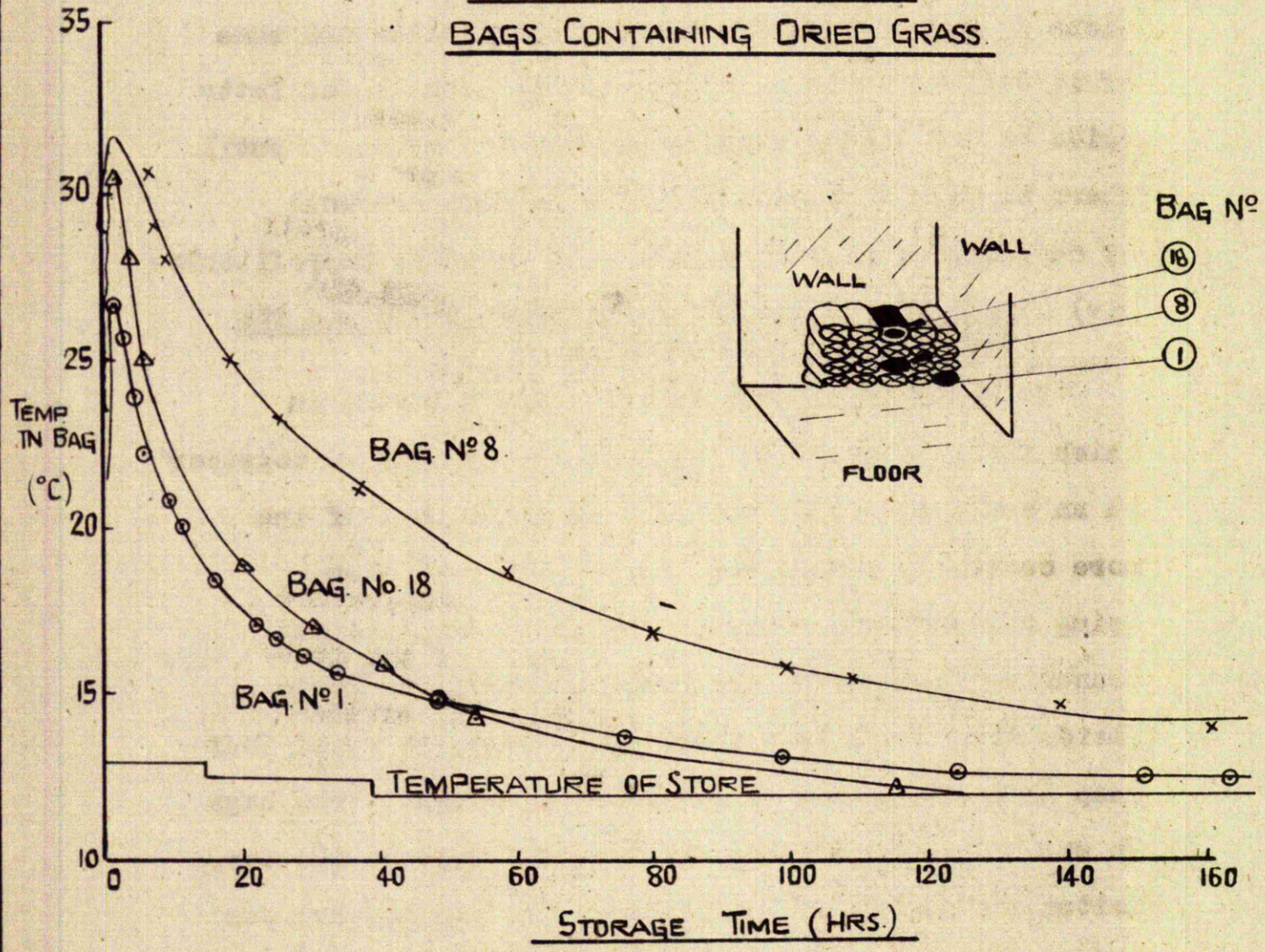
It is interesting to speculate on the course of the chemical changes which result in the gas compositions recorded. Starting from the assumption that an end product of β-carotene oxidation is β-ionone and that all the remaining carbon atoms ultimately form carbon dioxide as in the following system:-



it can be calculated that 1 mg. carotene thus oxidised requires 0.79 ml. O_2 and liberates 0.58 ml. CO_2 (N.T.P.). If any of the data concerning oxygen absorption is considered it can be shown that in no case does the

FIG. 21.

COOLING RATE OF STORED BAGS CONTAINING DRIED GRASS.



oxygen taken up by the carotene account on this basis for more than about 20% of the total removed from the air in the can. Even if the course of the reaction of carotene oxidation is vastly different from that suggested, as well it may be, it does not appear likely alone to account for all the oxygen absorbed and some of it will be taken up by the highly unsaturated fatty acids of the lipins present (46) in the grass. Until there is more knowledge of the breakdown products of carotene further speculation is probably unprofitable.

(iv) Rate of cooling of bagged dried grass and its effect on carotene content.

It was noticed that during a day's drying in which fifty to sixty 56 lb. bags may be stacked together in an enclosed place, the rate at which some of the more centrally placed bags cooled was very slow. Using a thermionic resistance-type of temperature measuring instrument, the temperature of the grass inside three bags in a stack of 20 bags, arranged four deep in a single row of five, was measured. The bags in which the temperature was recorded were at the top, bottom and middle of the stack. The top bag had one side cooled by the air, the bottom bag had one side cooled by a concrete floor while the centre bag was surrounded on all sides except at the mouth. Figure 21 is a record of the rate of cooling and Table 28 shows the carotene content of the grass at the centre of the three bags at the time when the temperature within the

Table 28.

Effect of cooling rate on carotene content.

Position of bag	Max. temperature recorded (°C.)	Days to reach air temperature	Carotene Content (mg./kg. dry wt.)
Bottom	27	3	226
Middle	30	9	230
Top	31	3	239

bag had reached that of the air in the store. Within the accuracy of the determination there was no appreciable difference in the carotene contents of the grass in the three bags at the start of the experiment and, as can be seen from the table, the centre bag had not suffered from being held for two days above 20°C.

DISCUSSION.

The data presented in Part 4 deal with problems arising from the preparation of dried grass, its storage and preservation.

Partial drying in the field, or wilting, has an economic significance but is unfortunately associated with high losses in carotene and these, as might be expected, are greater in warm sunny weather. From the results of the wilting trials it is suggested that field wilting for more than 1-2 hours causes too great a loss of carotene if a dried grass high in this constituent is desired.

The losses in carotene in freshly cut grass are partly due to enzymes present in the plant tissues, a theory first proposed by Hauge and Aitkenhead (24) and later confirmed by Hauge (23) and Guilbert (20). Waugh (54), however, suggested that in addition to enzymatic systems present some non-enzymatic destruction also occurs. Mitchell and Hauge (37) showed that the carotene destroying system in alfalfa was a

lipoxidase, and that the enzyme could be inactivated by heat. The degree of inactivation was shown to be a function of time and temperature, complete destruction being effected between 90 and 100°C. within ten seconds. It can be seen from the results presented here that prolonged field drying, even under unfavourable weather conditions, may destroy as much as 60% of the carotene with correspondingly greater losses under brighter conditions. These observations are in agreement with those of Greenhill(18) and Fagan and Ashton (12).

Russell (43) found that artificially dried alfalfa in addition to retaining its colour, contained seven times as much vitamin A as the leaves from hay naturally dried. In the present work digestibility trials have not been carried out, but it is of interest to quote the important results of Watson and Ferguson (59). These authors found that the digestibility and feeding value of artificially dried grass was of a high order and compared well with that of fresh grass but that too high a temperature of drying reduced the digestibility of the final product.

In the present trials comparing a commercial and laboratory drier very little difference in the carotene values of the dried grass was found. The slightly higher loss in the grass dried in the laboratory may have been due to the larger volume of air passing through it but the results in general, agree well with

those found by Watson (56). Whilst the plant material is losing moisture it is likely that the carotene losses will be very low because the evaporation of water prevents the cell structure of the grass from reaching the temperature of the drying air, but if the grass remains in the drier for any length of time when dry, a rapid loss of green colour ensues and the carotene is almost entirely lost.

The three-ply brown paper bags used commercially for dried grass have been shown to be capable of preventing any loss of carotene due to light although no doubt other light-tight material would be equally suitable. Kon and Thompson (30) have shown that there was no difference in carotene losses when either paper or jute bags were used. In the present work even when the dried grass either in the form of powder or bales was unprotected from sunlight, only a thin surface layer was bleached. It is of interest in this connection, that the effect of ultra violet light on the carotene in dried grass has been shown to be negligible by both Hauge and Aitkenhead (24), and Guilbert (20). Nevertheless even after ten weeks, at normal temperatures considerable losses of carotene in the baled grass had occurred, particularly in the looser parts of the bales, which can only be the result of atmospheric oxidation. These results are supported by Watson's (57) observations that storage under pressure reduced the carotene losses in dried

grass. Kane and Shinn (29) found losses to the extent of 36-40% during summer months and Smith (45) reported that baled lucerne hay lost 50% of its vitamin A potency during storage from August to November, whereas during the winter months negligible amounts were lost. Wiseman, Kane and Cary (63) calculated that 6.5% per month was lost during summer months, at mean daily temperatures of 70°F. from baled alfalfa. Guilbert (20) stated that temperature was the most important factor in carotene loss from dried forage and that storage at 0°C prevented the loss. Wall & Kelly (53) dealing with alfalfa meal and carotene extracts found over the temperature range of 0°C. to 37°C. maximum losses at 37.5° and minimum losses at 5°C. Dutton (10) dealing with dehydrated carrots stored at 98° and 120°F. in CO_2 found negligible losses in carotene and suggested that partial destruction of carotene at elevated temperatures created conditions unfavourable to further oxidation. Halverson and Hart (22), however, are of the opinion that the chief factor in the preservation of carotene in dried grass is a restoration of more rapid respiratory enzyme action, with utilisation of oxygen and formation of CO_2. They also stated that more destruction of the carotene oxidising enzyme lipoxidase would not lead to carotene preservation unless oxygen was excluded. This seems the more likely explanation, and it has been found in the present work that

increased availability of oxygen materially assists the destruction of carotene in stored dried grass although the consumption of oxygen was not directly proportional either to carotene loss or oxygen availability. Hoffman (25) found that in cans of alfalfa containing air, 57% of the carotene was lost in 112 days, whilst meal stored in atmospheres containing 94.7% nitrogen and 5.3% oxygen, and 99.7% nitrogen and 0.3% oxygen over the same period only 9 and 0.4% carotene were lost. He also found that carbon dioxide prevented carotene loss and that the permissible oxygen content of the storage atmosphere at the start of the storage period should be about 6%. The present work confirms these results but would fix the lower limit of oxygen content at 0.1 ml./g. of dried grass. Any process which materially reduces the oxygen content of the container will assist in carotene preservation.

Taylor & Russell (49) found that little loss occurred in alfalfa meal kept at low temperatures and pressures whereas storage in the dark at normal temperature and pressure resulted in high losses.

In conclusion it may be stated that, although commercially difficult to apply, storage conditions for dried grass in which a high carotene content is desired should be as near 0^{o}C. as possible, with some provision made for reducing the available oxygen, either by compression or gas packing, and should offer reasonable protection from light.

CONCLUSIONS.

From the foregoing section it would appear that the following conclusions may be drawn.

(1) Field wilting under conditions favourable to loss of moisture will cause great loss of carotene, particularly when the moisture is lost mainly as a result of hot sunny weather rather than wind.

(2) Grass dried under correct working conditions on a moving belt type of commercial machine should contain not less than 90% of the carotene content of the fresh grass.

(3) Increasing the length of drying time above thirty minutes at temperatures below 150°C. or above 10-15 minutes at 165 - 170°C. will cause considerable loss of carotene, as much as 56% being lost after 45 minutes at 165°C.

(4) Triple-ply brown paper bags should prevent any loss of carotene by photochemical action. In baled dried grass only a thin surface layer is likely to lose carotene because of exposure to sunlight, although air oxidation will cause progressive losses as storage time increases.

(5) In any closed container, if less than 0.1 ml. of oxygen per gramme of dried grass is available, there will be no appreciable loss of carotene; above 0.1 ml./g. the losses will increase with oxygen availability. This will be rapid during the first three months at normal temperatures and may

ultimately amount to 80% of the original carotene content.

(6) Reduction of the oxygen content of a dried grass container below 0.1 ml/g. will preserve the carotene content indefinitely.

(7) High storage temperatures will, in the absence of an inert atmosphere, lead to increased loss of carotene.

Appendix I.

Table I.

Section I. Yield of dry matter from each cutting.

D.C. = Date of cut, D.M. = dry matter lb./acre.

Treatment	A		B		C		D		E		F		G		H		I	
Cut No.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.
1	11/4	1803	11/4	606	11/5	1024	9/5	1528	17/4	1067	26/4	1564	19/4	1024	19/4	1116	10/5	554
2	8/5	1539	7/5	1552	8/5	1548	12/6	801	17/6	1735	26/5	1574	22/6	1618	20/6	1562	7/5	1748
3	7/6	1040	11/6	1542	11/6	896	9/7	859	12/6	984	2/7	529	5/7	878	21/7	788	7/6	1218
4	4/7	806	5/7	613	6/7	598	8/8	429	8/7	488	25/7	619	25/7	526	25/7	851	4/7	706
5	29/7	502	1/8	551	1/8	506	4/9	581	30/7	660	20/8	591	20/8	865	28/8	750	29/7	577
6	29/8	1085	4/9	806	4/9	574			26/8	559	25/9	488	1/10	498	3/10	867	16/8	710
7	2/10	536	17/10	226	17/10	155			25/9	708	30/10	159					1/10	661
8	30/10	136							29/10	555								
Total		6837		6815		4451		3965		6610		4855		6185		4935		6402

Appendix 1.

Table 2.

Series 4. Yield of dry matter from each cutting.

D.C. = date of cut. D.M. = dry matter lb./acre.

Treatment	A		B		C		D		E		F		G		H		I	
Cut No.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.	D.C.	D.M.
	14/5	2931	14/5	3124	14/5	3371	22/5	2227	23/5	1709	21/5	2891	21/5	2685	22/5	2142	9/5	2842
	17/6	1415	17/6	1338	20/6	1062	19/6	1228	19/6	1890	20/6	1114	18/6	1601	18/6	1561	15/6	1916
	18/7	823	18/7	855	2/8	743	12/8	553	25/7	566	26/7	750	19/7	695	26/7	687	9/7	1167
	15/8	1451	5/9	1082	15/10	862	16/10	585	5/9	1207	20/9	1097	20/8	1564	20/9	1198	12/8	1444
	5/10	840	30/10	361					15/10	757			16/10	774			5/10	988
Total		5460		6760		5938		4573		5629		5852		7269		5528		8557

[redacted]

Appendix 1.

Table 3.

Series 3 and 4. Percentage recovery of nitrogen from each treatment.

Treatment	A	B	C	D	E	F	G	H	I
Series 3	55.2	55.4	61.5	-	40.0	39.5	55.6	46.1	49.1
Series 4	61.5	70.7	80.5	74.3	45.4	55.3	57.2	64.4	65.3

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