INVESTIGATIONS INTO SOME EFFECTS OF CYSTEINE ADMINISTRATION TO THE NEW ZEALAND ROMNEY.

A thesis presented in partial fulfilment
of the requirements for the
Degree of Master of Agricultural Science
in Animal Science.

by

G. McL. Dryden
Massey University, New Zealand
January, 1968.

ACKNOWLEDGEMENTS.

I wish to thank my supervisors, Drs. F.R.M. Cockrem and G.A. Wickham for their encouragement and assistance over the course of this Masterate.

My thanks are also due to Mr. L. Hawthorne for his assistance in caring for the animals used in these experiments.

I also wish to acknowledge the assistance and advice given by members of the Sheep Husbandry, Veterinary Clinical Sciences and Dairy Husbandry Departments and Library staffs of Massey University, and by members of the staffs of the Palmerston North Hospital and the Department of Scientific and Industrial Research.

Gordon Dryden.

CONTENTS.

page.

CHAPTER 1. INTRODUCTION.

Historical Summary 1

The Present Work 2

Nomenclature 3

CHAPTER 2. REVIEW OF LITERATURE.

I. THE PROTEIN NUTRITION OF WOOL GROWTH.

Protein Metabolism in the Rumen 5

The Utilisation of Digested Protein 10

Protein Nutrition and Wool Production 11

II. THE METABOLISM OF THE SULPHUR CONTAINING AMINO-ACIDS.

The Intermediary Metabolism. 14

(a) the conversion of methionine to cysteine 14

(b) the interconversion of cysteine and cystine 15

(c) the main pathways of cystine and cysteine degradation 16

(d) the formation of bile acids 21

(e) the excretion of cyst(e)ine degradation products and the detoxification mechanism. 21

The Incorporation of Cyst(e)ine into wool and Hair Keratin. 22

page

III. THE EFFECT OF SULPHUR CONTAINING AMINO-ACID ADMINISTRATION TO THE SHEEP.

The Effect of Cyst(e)ine Administration on Wool Growth and Body Weight 28

The Effect of Sulphur Containing Amino-Acid Administration on the Character of the Wool Grown 34

(a) the chemical composition and topography of the wool fibre 34

(b) fibre diameter 35

(c) the physical properties of the fibre 35

CHAPTER 3 METHODS.

Introduction 37

Animals 37

Digestibility Determinations 37

(a) collection 38

(b) nitrogen analyses 39

(c) dry matter determinations 39

Feeding Regimes 39

Cysteine Experiments 40

(a) dose level response experiment 40

(b) protein level experiment 42

Wool Sampling and Measurement Methods 42

Methods of Data Analysis 45

(a) dose level response experiment 45

(b) protein level experiment 46

CHAPTER 4. RESULTS.

Dose Level Response Experiment. 51

(a) wool growth rate response 52

(b) fibre diameter response 53

Protein Level Experiment 54

(a) wool growth rate response 54

(b) fibre diameter response 57

(c) the effect of cysteine infusion on the digestibility of the basal feed 58

(d) the effect of cysteine infusion on liveweight 58

CHAPTER 5 DISCUSSION.

General 59

Experimental Results 61

The Mechanism of the Cysteine Effect 67

SUMMARY 72

BIBLIOGRAPHY 74

ACKNOWLEDGEMENTS i.

LIST OF TABLES v.

LIST OF FIGURES viii.

LIST OF PLATES x.

ERRATA x.

page

LIST OF TABLES.

(Note: 'f' means 'facing' throughout).

2.1. Sulphur Containing Amino-Acid Concentrations in the Rumen Contents. 9

2.2. Occurrence of Label in Wool Fibres after ^{35}S cystine Injection. 24

2.3. The Effect of Sulphur Containing Amino-Acid Dosage on Wool Production. 32

2.4. Liveweight Gain Response to Sulphur Containing Amino-Acid Administration. 33

3.1. Timetable of Events over the Experimental Periods. 37f.

3.2. Allottment of Treatments in the Protein Level Experiment. 41

3.3. Sampling Rotation During the Dose Level Response Post-experimental Period. 44

3.4. Sampling Rotation for the Protein Level Experiment. 44

3.5. Notation Used in Computations for Analysis of Variance Sums of Squares. 49

4.1. Mean Daily Wool Growth from Patch 2. 52f

page

4.2. Analysis of Variance of Data from 15 Hune Sampling 52f

4.3. Analysis of Covariance of Data from 15 June Sampling. 52f

4.4. Mean Daily Wool Growth from Right Mid-Side Patch. 54f

4.5. Analysis of Variance of Data Taken at the End of the Infusion Period. 55f

4.6. Analysis of Covariance of Data Taken at the End of the Infusion Period. 55f

4.7. Test of Difference Between Subclasses. 55f

4.8. Analysis of Covariance of Data Taken at the End of the Post-experimental Period. 55f

4.9. Adjusted Means and Multiple Range Tests. 55f

4.10 Showing the Subclass Means of Each Body Region and Their Ranking. 56f

4.11. Regressions of Mean Fibre Diameter against Wool Growth Rate for Data Taken at 7 Sept. 57f

4.12. Regressions of Mean Fibre Diameter against Wool Growth Rate for Data Taken at 28 Sept. 57f

4.13. Mean Crude Protein and Dry Matter Digestibilities. 58f

4.14. Analysis of Variance of Crude Protein Digestibility Data. 58f

4.15. Analysis of Variance of Dry Matter Digestibility Data. 58f

LIST OF FIGURES. page

2.1. Digestion of Protein in the Rumen 7

2.2. Proposed Pathways of Microbial Synthesis of Cysteine from Sulphate. 10

2.3. Flow Chart of the More Important Reactions of Cyst(e)ine. 21f

2.4. Occurrence of Label in Plasma. 22f

2.5. Label Concentration Changes over Time. 23f

3.1. The Relationship Between the Two Determinations Used to Make Up the Protein Level Control Wool Growth Rate Values. 43f

3.2. The Separation Of the Subclass Wool Growth Rates Given by the Control Values Used. 43f

3.3. Sampling Rotation for the Dose Level Response Experiment. 44

4.1. Wool Growth Rate Responses to Cysteine Infusion. 52f

4.2. Fibre Diameter Response to Cysteine Infusion 53f

4.3. Relationship Between Sample Standard Deviations and Fibre Diameters. 54f

page

4.4. Wool Growth Rate Responses Obtained in the Protein Level Experiment. 54f

4.5. Fibre Diameter Responses Obtained in the Protein Level Experiment. 57f

4.6. Fibre Diameters and Their Relationship to Their Sample Standard Deviations. 58f

4.7. Liveweight Responses to Cysteine Infusion. 58f

5.1. Proposed Flow Chart for Abomasaly Infused Cysteine. 59

5.2. Proposed Flow Chart for Venous Infused Cysteine. 60

5.3. The Metabolism of Cyst(e)ine for Wool Growth. 71f

page

LIST OF PLATES.

3.1. The Metabolism Crate Showing Collection and Infusion Apparatus. 40f

3.2. The Catheter in Position in the Jugular Vein. 40f

3.3. The 'Tuta' Infusion Apparatus with Catheter Attached. 40f

ERRATA.

page	line	
6	1	not a new paragraph.
22	22	'Eagle, 1960' should read 'Eagle, et al., 1960'.
59	Fig.(5.1)	no arrow from 'protoplasmic protein to 'urine'.
71	3	'De Bevasques' should read 'De Bersaques'.
72	19	'high protein-saline' should read 'high protein-saline subclass'.

CHAPTER 1. INTRODUCTION.

Historical Summary:

The first investigations into the effect of sulphur and sulphur compounds on wool production were carried out in the 1930's by Steyn (1931; 1933), Pierce (1933), Du Toit, *et al.* (1935), Marston (1932) and others, who fed elemental sulphur and sulphates to sheep in an effort to raise their wool production. Although contemporary work had shown that the hair growth rate of mice could be increased by the feeding of sulphur compounds the work with sheep was notably unsuccessful. Marston (1935) reviewed the state of knowledge of the sulphur nutrition of the sheep, was impressed by the apparent discrepancy between the cystine content of wool (about 11%) and pasture (about 1.5%), and concluded that the animal must synthesise sulphur containing amino-acids in its body. He administered sulphur containing amino-acids in various ways and although he obtained some increases in wool growth his experiments were inconclusive. No further research into the sulphur nutrition of the sheep was undertaken for some two decades.

Concurrently with the investigations into sulphur nutrition research into the effects of the plane of nutrition and dietary protein and energy levels was being conducted. It was found (Marston, 1948; Ferguson, Carter and Hardy, 1949; Daly and Carter, 1955; Ferguson, 1967) that an increased plane of nutrition caused an increase in wool production. In an attempt to define

the feed factor or factors responsible for this increase, isocaloric diets containing different amounts of protein were fed but it was found that when the protein content of the diet was increased to about 10% (the level at which the rumen micro-organisims cease a net synthesis of protein) any further increase in the dietary crude protein content had little or no effect on wool production. It was accordingly thought that wool production was limited by the amount of energy precursors in the diet. Results were obtained which indicated that wool production increased when the energy content of iso-amino diets increased (see Ferguson, 1959) but the experiments were again inconclusive.

As knowledge of the biochemistry of the rumen advanced it was realised that the failure of the earlier work in protein and sulphur nutrition to increase wool production was probably due to the intervention of the rumen micro-organisms and a consequent failure of the various experimental treatments to alter the nature and volume of amino-acids reaching the portal blood. Reis and Schinkel (1963) by-passed the rumen by infusing protein (casein in this case) into the abomasum and obtained wool production increases of up to 130%. Subsequent investigation aimed at determining more exactly the protein constituent(s) responsible for the increase showed that abomasal cysteine infusion increased wool production more than the infusion of gelatin supplying an equivalent amount of amino-nitrogen. It was concluded that the sulphur containing amino-acid content of the diets fed was probably a limiting factor for wool growth.

The Present Work:

Up to this date, all sulphur containing amino-acid and protein

infusion work had been done in Australia using Australian Merinos. It was decided to conduct a similar experiment to see whether cysteine infusion had a similar effect on the wool growth rate of New Zealand Romneys.

The work described in this thesis consists of two parts:-

(i) a preliminary experiment was conducted to see if the Romney wool growth rate responded to cysteine infusion, and if so, at what dose levels. A first investigation of the wool factors involved was also made.

(ii) having found that the Romney did respond to cysteine treatment a second experiment was carried out to determine more exactly the wool factors involved in the response and to see if the response was altered by the protein content of the basal diet. The effect of cysteine administration on the digestibility of basal diet was also examined.

The fact that the feed factor causing the wool growth rate increase was an amino-acid, not protein *per se*, allowed the use of intravenous infusion, a simpler technique than abomasal infusion. It is felt that the interpretation of the results of the present experiments is not greatly different from that of the abomasal infusion experiments as in both cases the amino-acid is transported via the venous blood to the sites of its utilisation in the body. A more detailed discussion of this problem is given in Chapter 5.

Nomenclature:

The term 'cyst(e)ine' is used throughout this thesis as a

general term for cysteine and cystine when it is not necessary to distinguish between the two.

The terminology used in Part II of Chapter 2 to describe the microstructure of the wool fibre is that of Mercer, *et al.* (1964).

The term 'thiol' refers to the -SH group, while 'dithiol' refers to the S-S configuration.

CHAPTER 2. REVIEW OF LITERATURE.

I THE PROTEIN NUTRITION OF WOOL GROWTH.

Protein Metabolism in the Rumen:

The digestion of protein in the sheep takes place in two phases, microbial and mammalian, and the importance of the microbial phase depends largely on the quality and dietary concentration of protein. When the dietary protein content is below about 10% the diet does not appear to meet the micro-organisms protein requirements and it is considered (Hamilton, et al. 1948; Burroughs, et al. 1951) that in this situation 60% to 80% of the protein digested by the animal in the abomasum is microbial protein which has been synthesised from non-protein nitrogen. Evidence supporting this view is given by Johnson, et al. (1942; 1944), Kehar and Mukhergee (1949), McNaught, et al. (1950) and Ellis, et al. (1956) who have shown that for ruminants, the amino-nitrogen absorbed by the animal has a biological value of about 70% when the proportion of crude protein in the diet is below 7% to 10%. This indicates that in these conditions much of the amino-nitrogen absorbed by the animal is of microbial origin.

This synthesis is efficient and non-protein nitrogen can supply all of the animals protein requirement (Virtanen, 1966). It has been shown that urea can replace dietary protein for wool growth purposes and that the synthesis of protein from non-protein nitrogen is enhanced if inorganic sulphate is available (Thomas, et al. 1951).

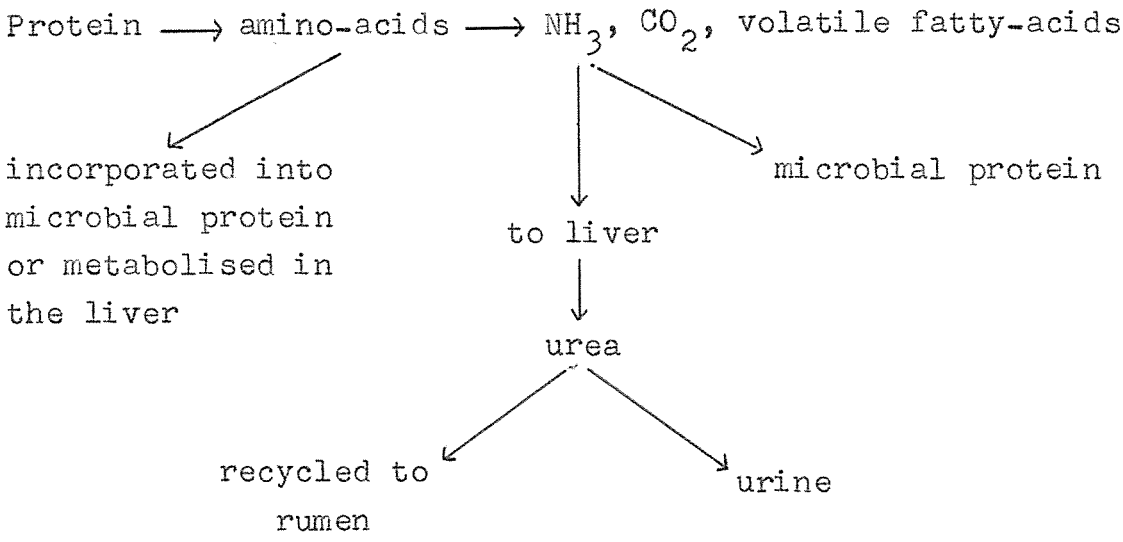
Starks et al. (1953; 1954) and Hale and Garrigus (1953) have also noticed the stimulatory effect of sulphate addition on wool growth and tracer studies have shown that sulphate can, in these circumstances, be converted to cystine and incorporated in wool.

It can be assumed, then, that in conditions of low dietary protein content the micro-organism will increase the amount of protein available to the animal, but it is considered (Chalmers, 1961) that the quantity of protein synthesised in the rumen of sheep fed a solely urea diet may not be sufficent to support good growth, the protein synthesis being limited by the availability of sulphur containing amino-acids.

When the dietary concentration of crude protein is above 10% to 12% little protein is synthesised from non-protein nitrogen as the micro-organisms obtain their requirements from dietary protein. However, in doing this, the micro-organisms hydrolyse the dietary protein and even in conditions of high dietary concentration most of the protein digested in the abomasum may be microbial in nature although the extent of the digestion of dietary protein can vary greatly (McDonald, 1954; McDonald and Hall, 1957).

The mechanism of protein hydrolysis in the rumen was first recognised by Sym (1938) and it is now known that most dietary protein undergoes the scheme of reactions shown in Fig. (2.1).

Fig. (2.1). Digestion of Protein in the Rumen (from Annison and Lewis, 1959).



The presence of carbohydrate, protein (or other nitrogen sources) and other substances (notably sulphur) affect digestion in the rumen, as does the physical form and solubility of the dietary proteins. Soya gluten, for example, is not hydrolysed in the presence of cellobiose, but in general the presence of easily available carbohydrate stimulates protein catabolism. The nitrogen / carbon ratio is of importance: Harris and Mitchell (1941) found that the addition of nitrogen to poor diets enhanced dry matter digestion, and Moir and Harris (1962) were able to positively correlate nitrogen intake with dry matter digestibility and hence rumen fermentative activity. Nitrogen need not be given *per os*, as Little and Mitchell (1967) obtained a similar enhancement of dry matter digestibility by the abomasal infusion of protein. It is known that both nitrogen and sulphur are recycled to the rumen and it is probably this mechanism which was responsible for this result.

In view of these observations, and considering the topic of this thesis, the activity of the rumen micro-organisms on dietary sulphur containing amino-acids is of interest. The pathways described in Part II of this Chapter for anaerobic cysteine breakdown are, generally, those existing in the rumen micro-organisms. Tarr (1933) found that Proteus vulgaris produced H_2S from cysteine degradation thus indicating the presence of the cysteine desulphydrase mechanism, Woods and Clifton (1937) found that Clostridium tetanomorphum ferments DL- serine and L - cysteine to H_2, CO_2, NH_3 and volatile fatty-acids (mainly acetate but also some formate) and Lewis and Elsden (1955) found that the organism LC (a gram negative coccus found in the sheeps rumen) degraded cysteine via the cysteine desulphydrase pathway. Sirotnak, et al. (1953) state that the volatile fatty-acids formed from cysteine and cystine hydrolysis consist mainly of acetate.

Under conditions of adequate protein there will be little net sulphur containing amino-acid synthesis. Rather, the situation will develop where the sulphur containing amino-acids present in the diet are hydrolysed and the hydrolysates used for the resynthesis of these amino-acids in microbial protein, e.g. see the results shown in Table (2.1).

Table (2.1). Sulphur Containing Amino-Acid Concentrations in the Rumen Contents. (from Weller, 1957).

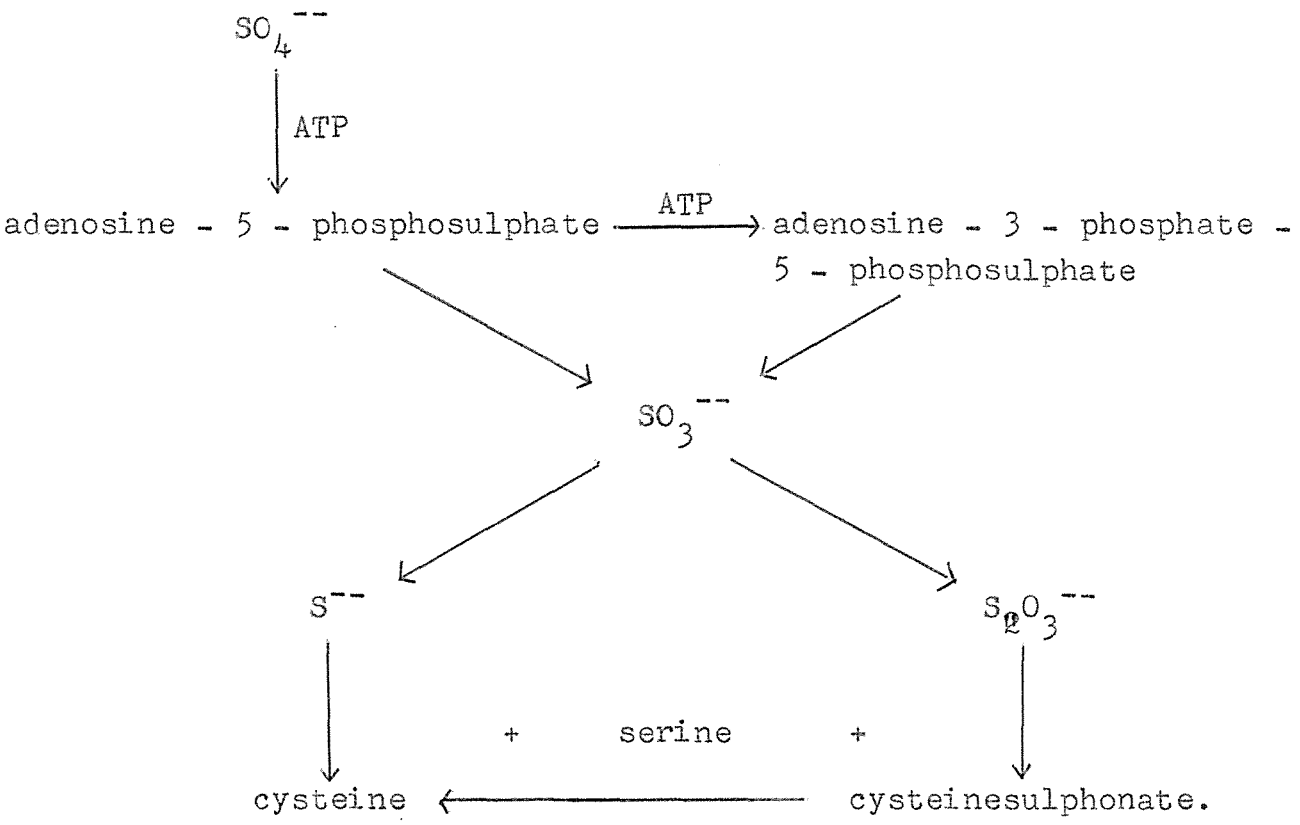
Amino-acid	Amino-acid as % of Total N.			
	micro-organisms		rumen digesta	herbage
	bacteria	protozoa		
cystine	0.7 to 0.8	1.1 to 1.3		1.1 to 1.6
methionine	1.5	1.0 to 1.4	0.5 to 0.9	1.2 to 1.6

Microbial sulphur will eventually become available to the animal but there will be an appreciable time lapse before this happens and some, at least, of the dietary sulphur containing amino-acid will be lost to the animal because of microbial desulphuration and production of H_2S.

When micro-organisms are grown in a medium deficient in sulphur containing amino-acids they will synthesise them from inorganic sulphate (Block, et al. 1951; Hale and Garrigus, 1953; Tsukamura, 1964). Meister (1965) has summarised the present knowledge of the microbial conversion of sulphate to cysteine in the scheme shown in Fig. (2.2).

Lewis (1954) showed that the optimum pH for sulphate reduction is 6.5 (i.e. that of the rumen) when H_2 gas is the hydrogen donor, and that pyruvate, formate, succinate, lactate, glucose and volatile fatty-acids will also act as hydrogen donors.

Fig. (2.2). Proposed Pathways of Microbial Synthesis of Cysteine from Sulphate (from Meister, 1965).



The Utilisation of Digested Protein:

The efficiency of utilisation of protein digested in the abomasum depends on its biological value and on the energy status of the animal.

The biological value of a protein is described (Cuthbertson, 1965) as:-

$$\frac{\text{absorbed nitrogen - excreted nitrogen}}{\text{total absorbed nitrogen}} \%$$

The biological value of microbial protein is high, probably because of its high essential amino-acid content. Even so, the addition of sulphur to the medium will enhance both microbial growth and the biological value of microbial protein (Williams and Moir, 1951; Holmes, et al. 1953). It is of interest that Hawley, et al. (1948) obtained a positive correlation of 0.972 between biological value and the ratio:

$$\frac{\text{creatinine nitrogen}}{\text{total urinary nitrogen}}$$

As methionine is a precursor of creatinine this suggests that the sulphur content of a protein may determine its biological value.

If an animals carbohydrate and fat intake does not satisfy its energy requirements then the amino-acids resulting from protein hydrolysis will be used for energy purposes. Unless a selective mechanism is proposed this will reduce the amount of cyst(e)ine available to the animal. Also, if sufficient energy for the incorporation of sulphur containing amino-acids into the animals tissues is not available these amino-acids will probably be excreted as sulphur compounds in the urine.

Protein Nutrition and Wool Production:

Once the digestive process is understood the apparently conflicting results of the earlier work in the protein nutrition of wool growth can be reconciled.

The earlier workers (Orr and Holm, 1931; Spottel, 1933;

Fraser and Roberts, 1933; Bowstead and Larose, 1938; Leroy and Chartelet 1949; Van Horn, *et al*. 1950; 1951) found that no increase in wool growth rate was obtained when protein supplements were given above a normal level of protein feeding. Van Horn, *et al*. (1950; 1951) obtained no increase even when the dietary protein content was raised to 39%. Slen and Whiting (1952) fed differing amounts of protein in isocaloric rations and obtained wool growth rate increases when the protein level was raised from 7% to 10% but little increase was obtained as the level was raised above 10%. Marston (1932), however did obtain wool growth increases after feeding a dried blood supplement, but the basal dietary protein level was probably less than 10%. Ferguson (1959) performed an experiment similar to that of Slen and Whiting (1952) and obtained similar results. He concluded that energy was the factor limiting wool production but this theory was disproved by subsequent Australian work (cited by Fraser, 1967) using isocaloric rations derived from different foodstuffs.

Coetzee and Pieterse (1966) found that the type of protein fed had little effect on wool growth rate and Little and Mitchell (1967) found that different proteins had similar effects on dry matter digestibility and nitrogen retention when given *per os*. Lofgreen *et al*. (1947) and Williams and Moir (1951), however, obtained different wool growth rates when they fed different proteins to lambs. It is possible that the lambs did not have developed rumens and consequently responded as monogastric animals.

While no response to increased protein level was obtained when the level of dietary protein was about 10%, there is evidence that the wool growth rate does respond to increases in total protein intake. For example, Ferguson (1959) found no difference in wool production between sheep fed similar amounts of rations containing 7.5% and 29% crude protein but did obtain differences between animals fed 500 gm. and 1400 gm. per day of rations containg both levels of protein.

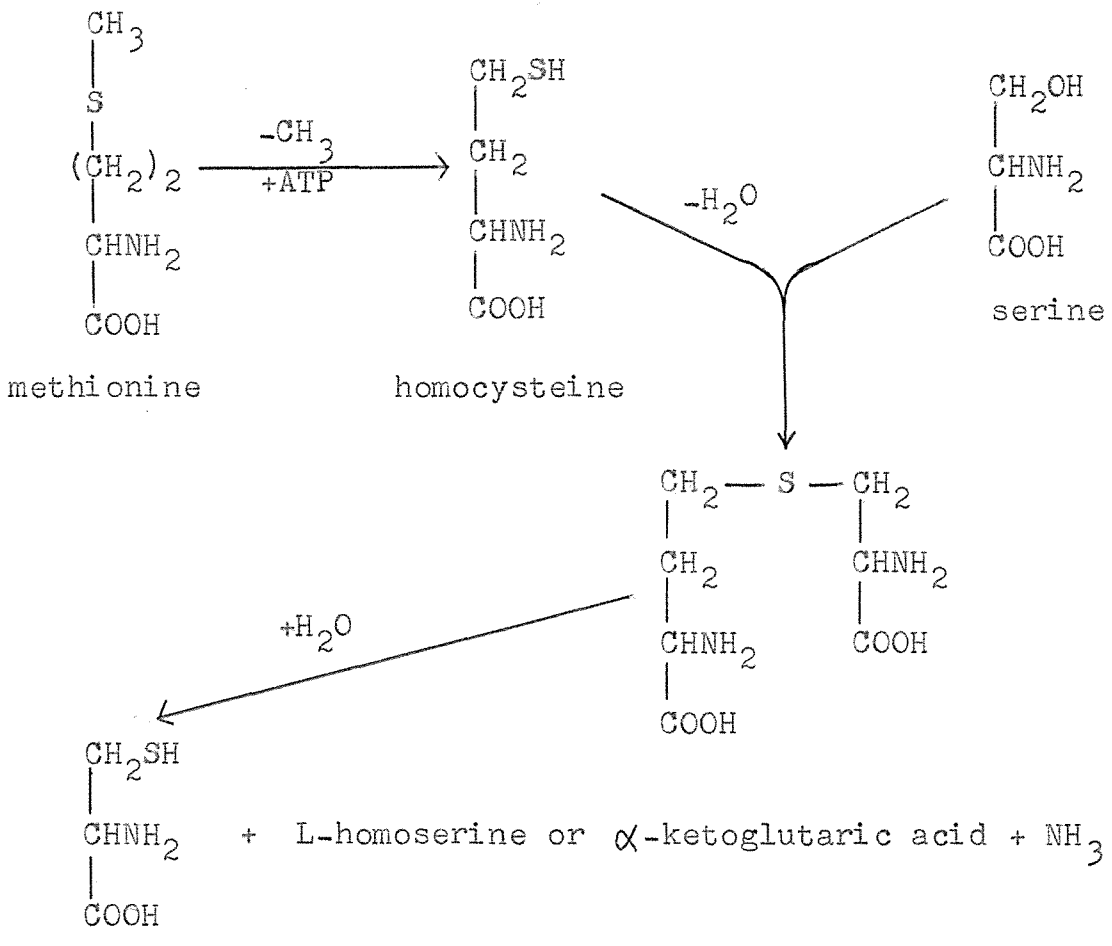
Recent Australian work (cited by Fraser, 1967) has suggested that the feeding of different proteins may not stimulate wool production to the same extent (see above). However, as it was necessary to feed the various diets at different levels in order to obtain iso-caloric intakes, it is possible that the effect of protein source was confounded with that of protein intake level.

II THE METABOLISM OF THE SULPHUR CONTAINING AMINO ACIDS.

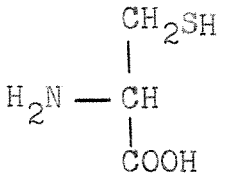
The Intermediary Metabolism:

The following section contains brief descriptions of those metabolic pathways of importance in the utilisation of administered cyst(e)ine and methionine for wool production, and the more important pathways by which these amino-acids are metabolised in the body.

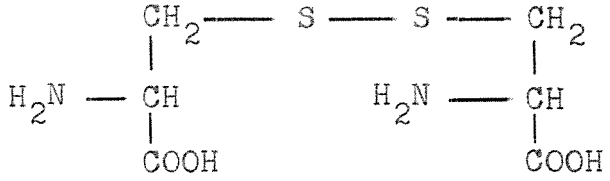
(a) the conversion of methionine to cysteine: this involved the addition of the methionine sulphur to the carbon skeleton of serine. (du Vigneaud, et al. 1944; Stetten, 1942). Rachele, et al. (1950) give the pathway as:-



(b) <u>the interconversion of cystine and cysteine</u>: as can be seen from the formulae, cysteine is the reduced half-residue of cystine.



L-cysteine



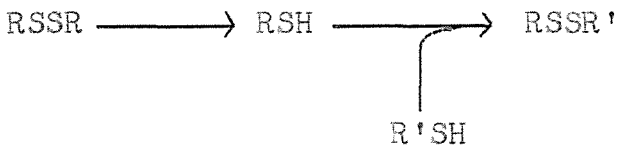
L-cystine

In neutral or alkaline solutions cysteine is oxidised by air to cystine.

The conversion of cysteine to cystine is catalysed by cytochrome oxidase with cytochrome c as a cofactor (Keilin, 1930).

This redox reaction is an important one. Not only does it provide an important electron accepting and / or donating system but it provides the means of activating enzymes such as oxytocin and vasopressin and of forming dithiol bridges between amino-acid chains such as are found in the insulin and keratin molecules.

The usual method of forming the dithiol bond is for this reaction to occur:



i.e. any existing dithiol bonds are reduced before combination with a sulphur ion of the second molecule. However, Jensen (1959) has noted that in some situations a more subtle formation

of a new dithiol configuration occurs. This reaction can be shown under acidic conditions <u>in vitro</u> as:

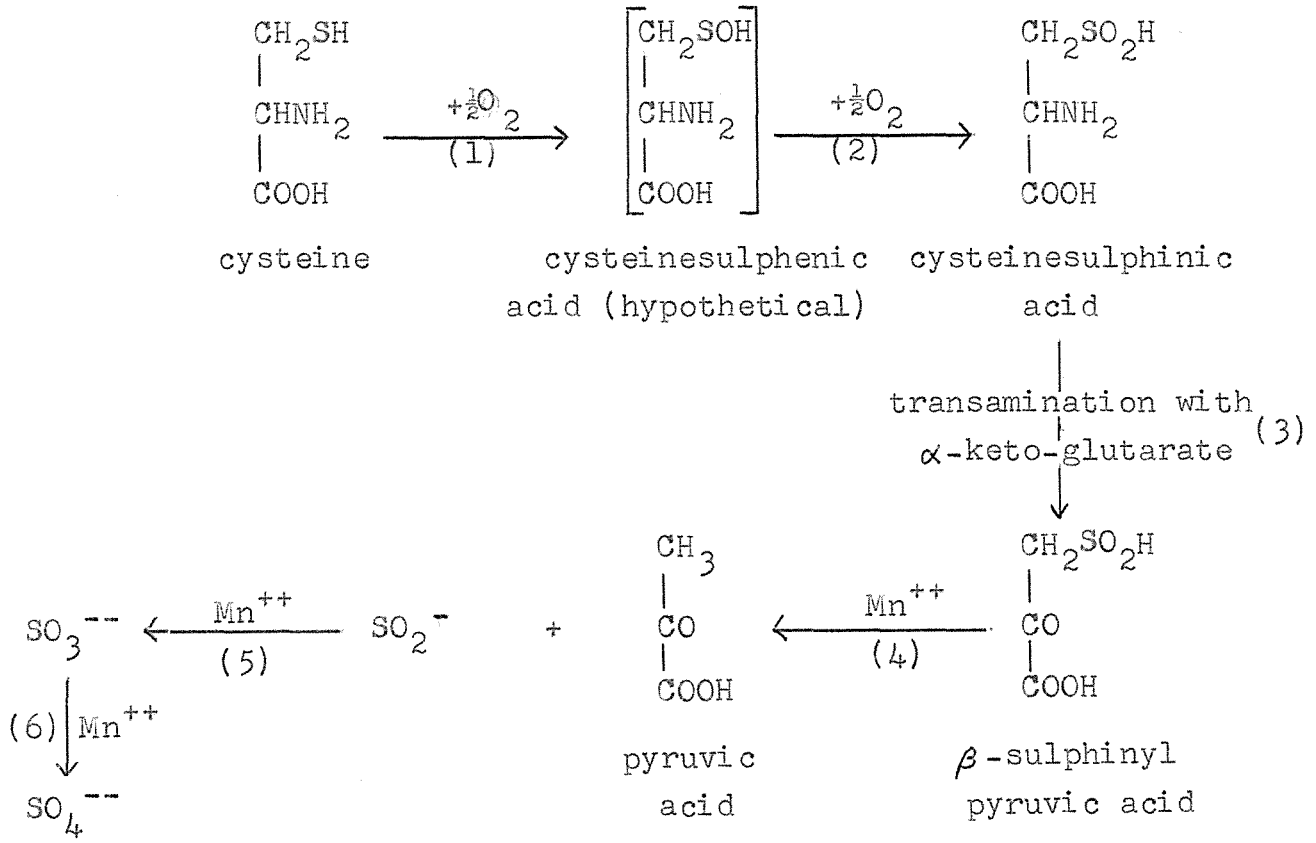
$$RSSR + HCl \rightleftharpoons RSCl + RSH$$

$$RS^{+}\,Cl^{-} + R'SSR' \rightleftharpoons RSSR' + R'SCl$$

and a chain reaction is set up with a little of the RSCl radicle available after each dithiol bond formation to instigate the formation of the next.

(c) <u>the main pathways of cystine and cysteine degradation</u>: these are similar except for one path which is specific for cystine. They are shown below:

(i) aerobic degradation of cysteine (Medes and Floyd, 1942):

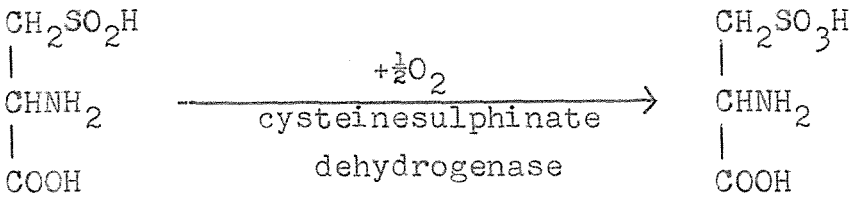


Reactions (5) and (6) are not enzymatic.

This pathway is quantitatively the more important one.

Kun (1961) suggests a possible formation of β- sulphonyl pyruvate from β- sulphinyl pyruvate. This would be excreted as such in animals but may, in micro-organism, undergo a transamination with glutamic acid forming cysteic acid.

Also, instead of step (3) as shown we may get:

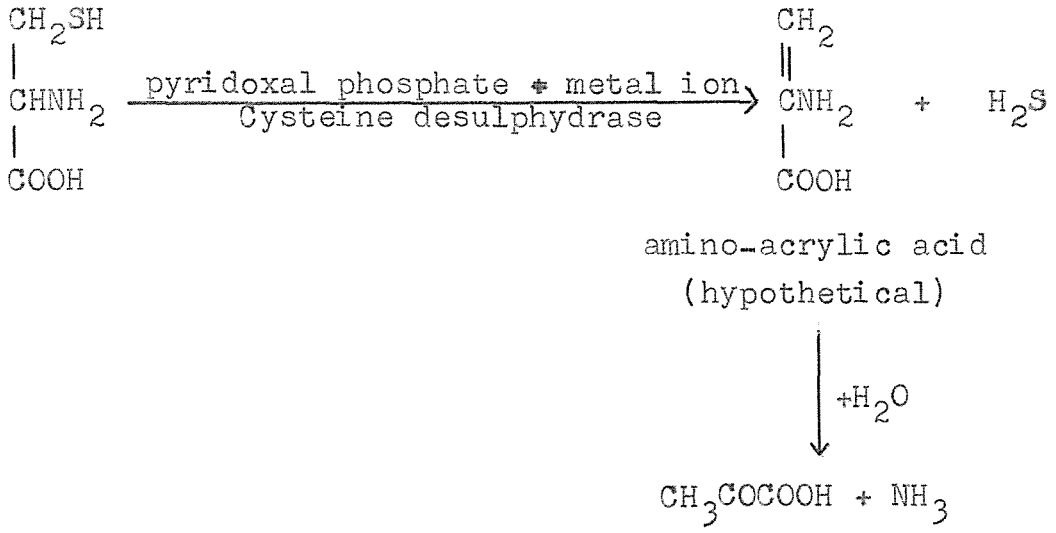


cysteic acid

Cysteic acid is then transaminated with α-keto-glutarate to form β-sulphinyl pyruvate (Cohen, 1940).

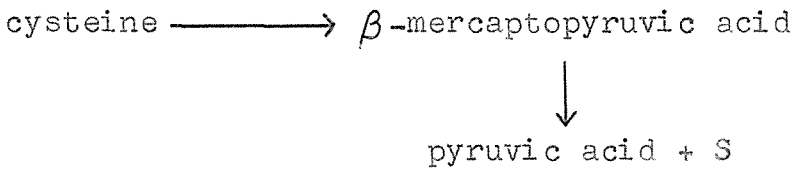
Chapeville, et al. (1956) suggest that reaction (4) may be reversible, allowing synthesis of amino-sulphur compounds from inorganic sulphate.

(ii) the anaerobic cysteine desulphydrase pathway: Smythe, (1942), Fromageot (1951) and Kalan and Ceithaml (1954) suggest this sequence of reactions:



Of the H_2S formed in the system, 75% to 85% appears in the urine as sulphate.

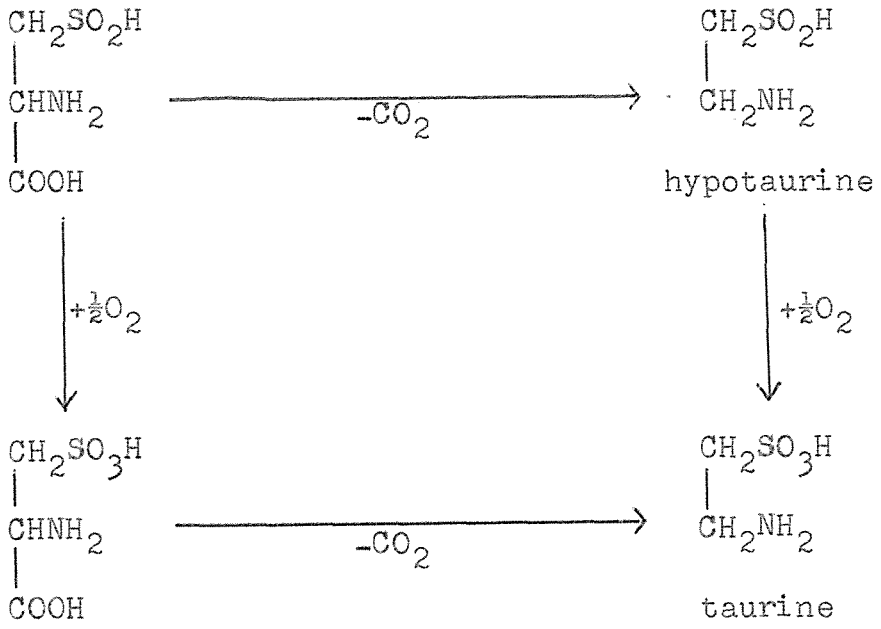
Meister (1965) suggests a transamination mechanism:



In the presence of excess cysteine, H_2S and cystine are formed. This pathway should, theoretically, produce alanine, H_2S and cystine but in practice we get NH_3, H_2S, pyruvate and cystine. It is thought that the deamination of glutamic acid (an intermediate) occurs, rather than the theoretical transamination.

It is possible (Meister, 1965) that other desulphydrase pathways exist.

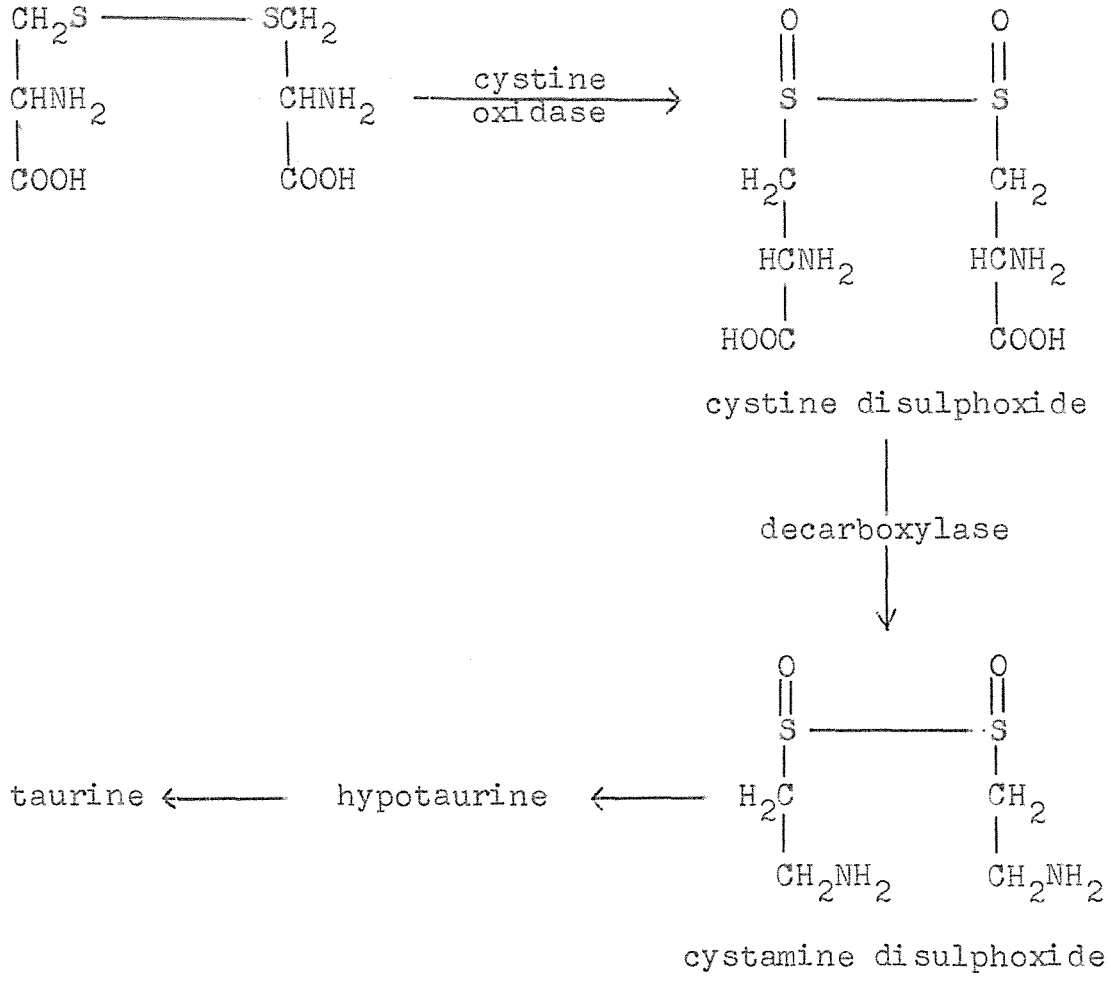
(iii) the formation of taurine can be summarised thus:



The route via hypotaurine is probably the preferred path (Cavallini, et al., 1955; Hope, 1955).

Several workers have found that taurine could not replace cystine in the diet of rats so it would seem that these pathways are irreversible.

(iv) a degradation pathway specific for cystine was proposed by Pirie (1934) and Medes (1939). More recent work (quoted by Meister, 1965) has supported this proposed path:



It is possible however, that the artificially synthesised 'cystine disulphoxide' is in fact the isomeric thiosulphonate compound $R\text{-}SO_2\text{-}S\text{-}R$ (Meister, 1965).

In all cases the eventual end-product of cyst(e)ine degradation is sulphate (which may be used in further metabolic processes or excreted) or taurine. The carbon skeleton is converted to pyruvate but, contrary to expectation, the sulphur containing amino-acids seems to be variably glycogenic, i.e. cause

the net production of energy, (Krebs, 1964). Little is known about the carbon metabolism of these amino-acids.

(d) the formation of bile acids: taurine, when conjugated with cholic acid, forms the bile acid taurocholic acid. The administration of sulphur containing amino-acids may cause a shift from glycocholate to taurocholate, but it is unlikely to cause the formation of more taurocholate unless cholic acid is also given, otherwise the excess sulphur will appear in the neutral sulphur of the urine.

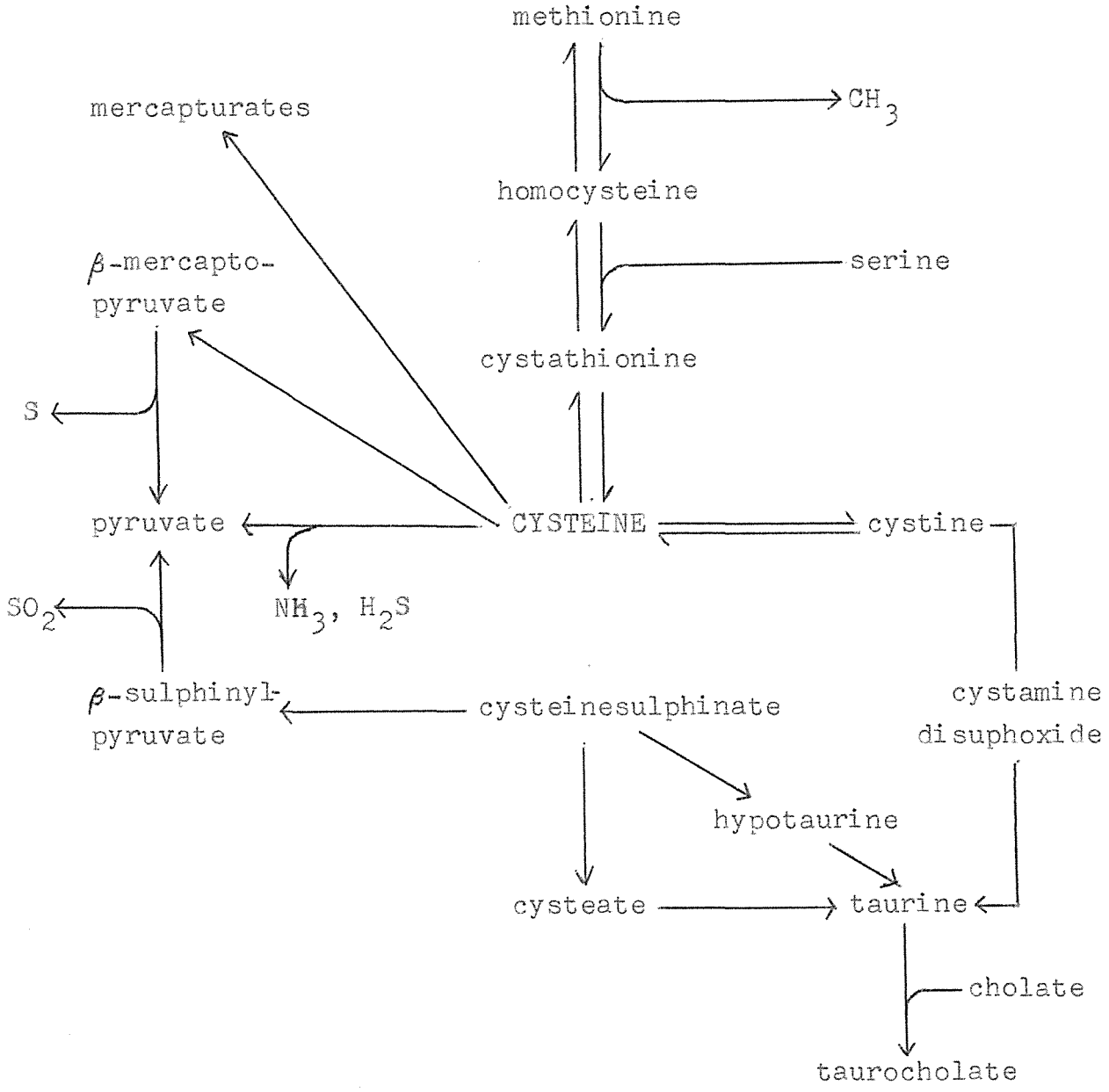
(e) the excretion of cyst(e)ine degradation products and the detoxification mechanism: the sulphur content of urine can be subdivided into inorganic and ethereal sulphates and neutral sulphur.

As was seen in section (c) a major end-product of cyst(e)ine degradation is sulphate which is excreted in the urine (Block, et al., 1965). The other component of the urinary sulphate fraction is ethereal sulphate; esters of H_2SO_4 formed between phenolic hydroxyl groups and sulphate. They have the general formula $R\text{-}O\text{-}SO_3^-$.

Neutral sulphur in the urine arises in two ways: from the detoxification of phenols, and from endogenous sulphur compounds which include cystine, cysteine, methionine, taurine and ethyl sulphur compounds.

A flow chart of the more important reactions is given in Fig. (2.3).

Fig. (2.3). A Summary of the Metabolism of Cysteine (adapted from Meister, 1965).



The Incorporation of Cyst(e)ine into Wool and Hair Keratin:

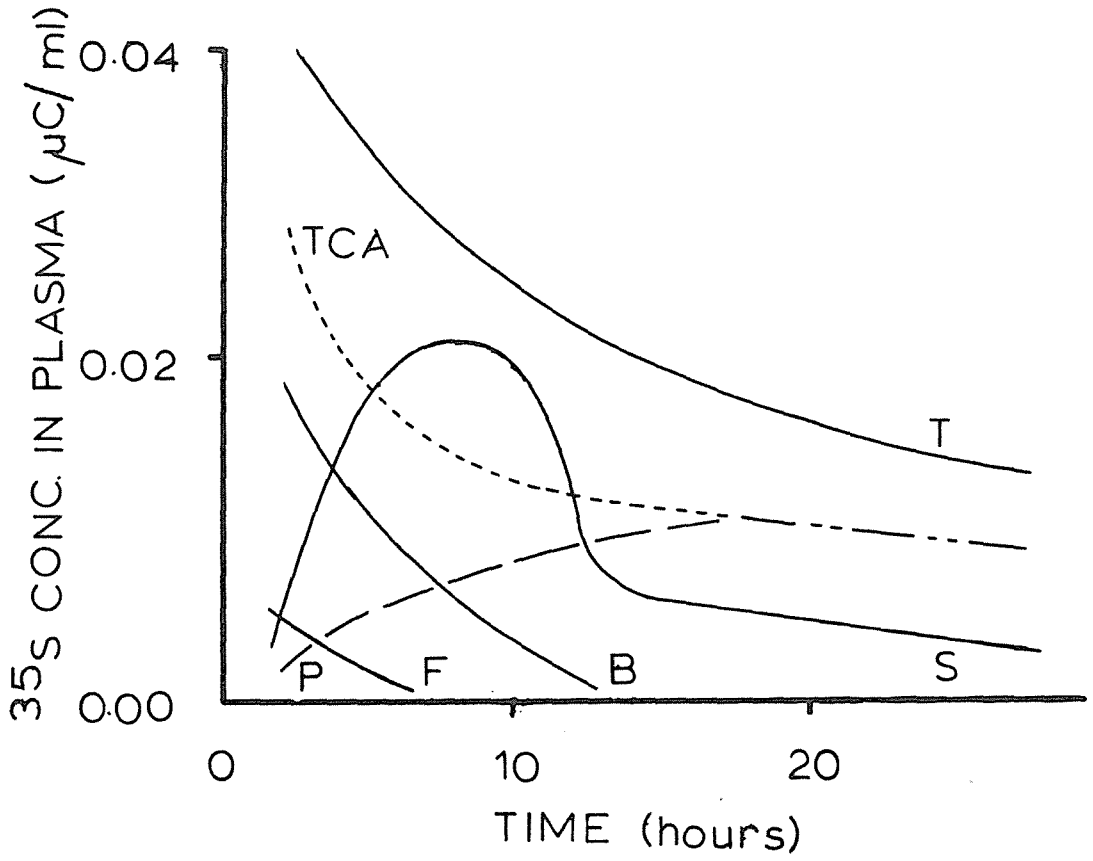
The incorporation of cyst(e)ine has been investigated mainly by the use of radioactive sulphur compounds. The injection of labelled cyst(e)ine that this method entails is analogous to the infusion experiments described in Part III and so the mechanisms elucidated by this technique will be those involved in the incorporation of infused cyst(e)ine.

Edwards (1954), Ryder (1958) and Downes, et al. (1964) have confirmed that the sulphur containing amino-acids are transported to the wool follicle by the blood and the mechanism of cyst(e)ine transport in the blood has been investigated by Downes. In his first experiment (Downes, 1961a) a Corriedale ewe was injected intravenously with ^{35}S cystine and the amount of ^{35}S present in various fractions of the plasma was measured. The results are shown in Fig. (2.4).

The 'bound' cystine was removed by $NaHSO_3$ (Downes, 1961a) or by mercapto-ethanol (Lee, et al., 1951) before the TCA precipitation. Lee, et al. (1951) and Samarina, et al. (1956) have mentioned that cystine can be bound to plasma protein by a dithiol bond which can be broken by these reagents, and Downes (1961b) showed that the 'bound' cystine was, in fact, bound in virtro to the plasma proteins by dithiol bonds (see Jensen, 1959; Eagle, 1960; Downes, et al., 1965).

Downes (1961a) showed that it is the free and dithiol bound cystine which is used in keratin synthesis although the relative importance of each could not be calculated.

FIG. (2.4.) OCCURRENCE OF LABEL IN PLASMA.



KEY	T	means	Total 35 S
	TCA	"	All 35 S cystine in plasma proteins
	B	"	35 S cystine bound to plasma proteins by dithiol bonds.
	P	"	35 S cystine bound to plasma proteins by peptide bonds.
	F	"	Inbound 35 S in plasma

He suggests (Downes, 1961b) that some of the administered excess cystine may form a pool in the blood plasma, rather than being degraded and excreted. Data indicating this is shown in Fig. (2.5).

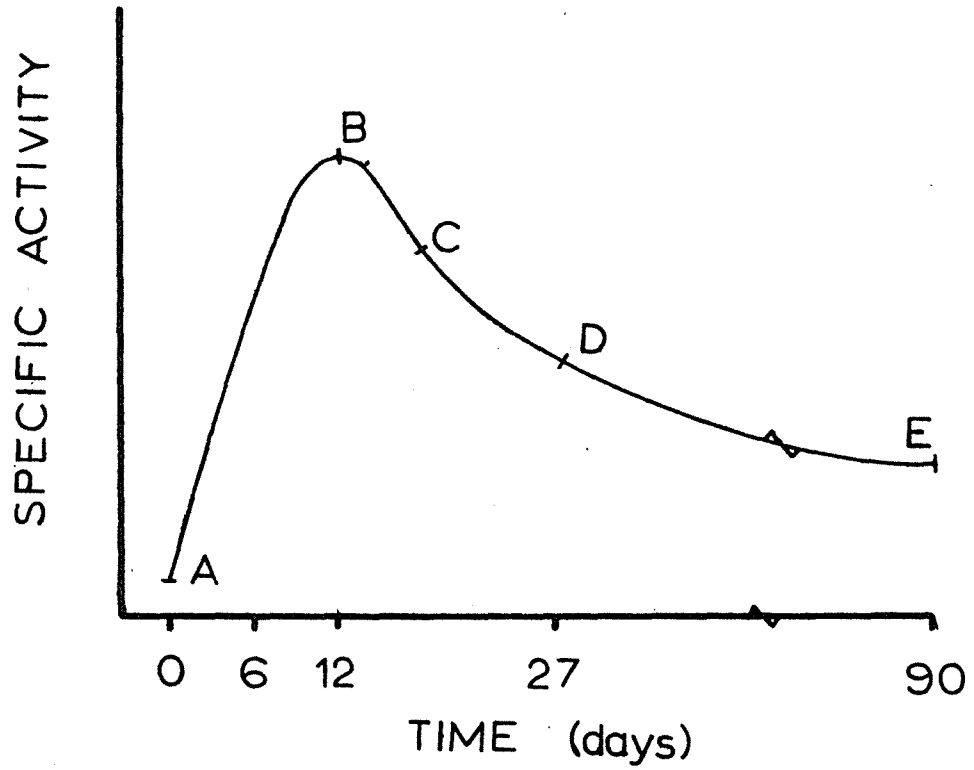
The rapid rise in specific activity in the first six days after ^{35}S cystine injection shows the rapid incorporation of a portion of the injected cystine. It would be expected, if all the injected cystine was incorporated into the wool grown, that the descending arm of the graph would mirror the ascending one, with a long tail indicating that some of the cystine had been introduced into the general sulphur metabolism. Downes suggests that a third curve exists (the portion CD of the graph) and that this indicates the presence of a cystine 'pool' bound by dithiol bonds to the plasma proteins.

The majority of sulphur incorporated into the fibre is in the form of either cystine or cysteine (Edwards, 1954; Downes, et al., 1964), administered methionine being first converted by the reactions already described.

Although the permeability of the follicle wall was questioned by King and Nicholls (1933) it is now considered that most of the sulphur containing amino-acids reaching the follicle enter it through the follicle wall and not through the papilla as was suggested by Burns and Clarkson (1949).

Bern (1954) and Bern, et al. (1955), Harkness and Bern (1957), Ryder (1958) and Downes, et al. (1962) have shown that an

FIG. (2.5.) LABEL CONCENTRATION CHANGES OVER TIME.



intravenous injection of ^{35}S cystine will cause label to appear at the suprabulbar and / or keratogenous regions while little appears in the bulb. A representative result is shown in Table (2.2).

Table (2.2). Occurrence of Label in Wool Fibres after ^{35}S Cystine Injection. (from Ryder, 1958).

Time	Occurrence of Label.
0.5 hours	peak in suprabulbar region.
8.0 hours	activity increased in the keratogenous zone and decreased in the suprabulbar region.
24.0 hours	activity in the keratinised fibre.

It is concluded that cyst(e)ine probably enters the follicle through the outer root sheath at the suprabulbar and / or the keratogenous regions, and then may diffuse throughout the fibre as Downes, et al. (1962) have found that radio-activity spreads downwards in the follicle as well as upwards, an observation which indicates that sulphur containing amino-acids are moved in the follicle by some means other than the growth of the fibre. It is suggested that the mechanism involved is the exchange reaction described by Jensen (1959).

It is not known whether infused cyst(e)ine is incorporated into the high-sulphur (matrix) fraction or the low-sulphur

(filamentous) fraction of the fibre. Ryder (1958) suggest that the appearance of activity in different areas of the follicle reflects the incorporation of ^{35}S cystine at the sites of greatest demand and as it appears that the low sulphur filament proteins are formed before the cell reaches the prekeratogenous zone (Mercer, 1961) this would suggest that the greatest demand for cyst(e)ine is for the production of the high-sulphur matrix protein. Gillespie, et al. (1964) noted a change in the proportion of high-sulphur protein to low-sulphur protein in wool grown under the influence of cystine infusion (see Part III) and on the basis of this suggested that the synthesis of at least part of the high-sulphur protein fraction is controlled by the availability of sulphur containing amino-acids. It has also been suggested that new high-sulphur proteins may form when excess cyst(e)ine is present.

On the other hand, Nakai (1964) has injected rats with ^{35}S cystine and electron microscopic examination of the autoradiograph appeared to indicate that the injected ^{35}S cystine was "directly and specifically incorporated into filaments in the hair cortex". Downes, et al. (1966) have also found radioactivity in the low-sulphur protein. They suggest possible explanations for this result:

(i) error.

(ii) some low-sulphur proteins may be synthesised in the upper follicle (Downes, 1961a found evidence of cyst(e)ine incorporation during the later stages of keratinisation).

(iii) incorporation of high-sulphur protein into low-sulphur helices (Crewther and Harrap, 1965).

(iv) the low-sulphur fraction may have been a constituent of a higher-sulphur protein formed after the ^{35}S cystine injection and broken down in the extraction process.

A further suggestion is that cystine may be exchanged with some other amino-acid previously incorporated into the keratin protein.

Administered excess cyst(e)ine may thus become a constituent of the low-sulphur proteins and a source of the dithiol bonds linking the α - helices, or be incorporated into the high-sulphur matrix protein. While the bulk of the evidence suggests that it is incorporated into the matrix this theory can not be proven or disproven until the mechanism of filament and matrix formation is elucidated.

Although Bern (1954) and Bern, et al. (1955) could find little ^{35}S in the follicle bulb after labelled cystine injection and Braun - Falco (1958) found almost no thiol groups in the papilla and fewer in the germinal tissue than in other parts of the follicle, Downes, et al. (1962) found radioactivity in all parts of the follicle at a time after the injection of ^{35}S cystine. Downes, et al. (1962) suggested that this may be due to the conversion of cystine to sulphate and the uptake of this sulphate in the bulb. This theory, if correct, could explain the increased growth rate of wool fibres after cyst(e)ine infusion as Schinkel (1962) and Short,

et al. (1965) have shown that the greater rate of wool growth obtained from sheep infused with 185 gm. casein per day was due largely to an increase in the rate of cell production in the germinal tissue, and it is known that sulphur is an important constituent of the cells mitotic apparatus (Stern, 1959; Mazia 1959; 1961). Sylven (1950) suggests that sulphate containing mucopolysaccharides may be implicated in keratinisation (possibly being inhibitory).

III. THE EFFECT OF SULPHUR CONTAINING AMINO-ACID ADMINISTRATION TO THE SHEEP.

The Effect of Cyst(e)ine Administration on Wool Growth and Body Weight:

Cystine, cysteine, methionine and inorganic sulphate and sulphur have been administered to sheep and other animals in order to study their effect on hair and wool growth and body weight. Two main factors affecting their utilisation have appeared: the degree of intervention of rumen micro-organisms, and the animals basal plane of nutrition.

Early experiments with rats showed that the stimulatory effect of cystine when fed to correct a cystine deficient diet (du Vigneaud, et al. 1932) was not due to its amino nitrogen content as an equivalent amount of amino nitrogen alone did not restore the rats growth rate.

Experiments by Evvard, et al. (1914), Hartwell (1921; 1925) and Smuts, et al. (1932) showed that rats fed a cystine deficient diet grew less hair than rats fed a normal diet and that this lack of growth was rectified by the feeding of cystine.

Steyn (1931; 1932) fed sheep 5 to 30 gm. sulphur per week and obtained increases in liveweight gain and wool production from this treatment. Pierce (1933) repeated this work using 2 gm. sulphur per day and continued the experiment for seven months but obtained no liveweight gain or wool growth rate

increases. He contrasted the nutritional states of his and Steyn's sheep and concluded that Steyn's result was due to the sheep being in a very poor nutritional state. However, as Pierce's sheep were fed a basal ration containing casein it is probable that their cystine requirements were met from the methionine content of the casein. Sneddon and Chamberlain (1933) performed an experiment similar to Pierce's with similar results, and Du Toit, et al. (1935) administered cystine and sulphur per os but obtained no effect on wool production or liveweight gain.

Marston (1935) fed Merino sheep diets on which they maintained body weight but grew only about 60% of their normal wool production (i.e. 30% of their total wool production potential: Marston's figures) and then:

(i) fed 1.0 gm. cystine per day and obtained improved nitrogen retention and increased wool production. About 80% of the added cystine was retained;

(ii) injected 1.5 gm. cysteine subcutaneously and obtained a 34% increase in wool growth rate. Two thirds of the cysteine was incorporated almost immediately and most of the remainder was incorporated over a subsequent period. The response lasted for some time;

(iii) added 1.0 gm. elemental sulphur to the diet but obtained little wool growth rate response; and,

(iv) injected methionine and cystine at doses calculated

to supply equal quantities of sulphur and obtained increases in wool growth with a 5% greater increase from methionine than cystine.

From this work, and the work of Thomas, et al. (1951), Hale and Garrigus (1953) and Starks, et al. (1953; 1954), it is obvious that the addition of inorganic sulphur will not improve the sulphur nutrition of the animal unless its basal diet is very low in protein-sulphur. Also, it can be seen that cyst(e)ine administration will increase the rate of liveweight gain and wool growth, and that, in general, this will occur when the cyst(e)ine is not subject to microbial action. The reasons for these observations and the probable mechanisms for the incorporation of inorganic sulphur and sulphate into protein have been discussed in Part II of this Chapter.

Reis and Schinkel (1961) found that the abomasal infusion of up to 55 gm. casein-nitrogen per day caused a 3% to 6% increase in the amount of wool produced each day. A rapid increase in fibre diameter accounted for half the observed increase in wool production.

Schinkel (1962) obtained increases in wool production of 150% from abomasal casein infusion and Short, et al. (1965) showed that most of this increase was the result of an increase in the cell proliferation rate in the wool follicles.

Reis and Schinkel (1963) fed sheep a basal diet providing

2.0 gm. cystine per day and compared the abomasal infusion of casein with some amino-acids. Two sheep given 60 gm. casein per day (equivalent in sulphur content to 1.75 gm. cystine) responded with wool growth rate increases of 84% and 102%. In comparison, 2.0 gm. cysteine per day caused increases of 25% to 75%, 2.46 gm. methionine per day caused an increase of 130%, while a mixture of glycine and glutamic acid having the same nitrogen content as the cysteine gave no response.

In a further experiment (Reis and Schinkel, 1964) the stimulatory effects of casein and cysteine on wool production were compared. Here, 60 gm. gelatin per day did not increase wool production, while 60 gm. casein per day doubled the rate of wool growth. The addition of cysteine and methionine to these regimes increased wool growth still further. These results were interpreted as showing that the increases in wool growth rate observed from casein infusion were largely due to the cyst(e)ine content of the casein. It is of interest that while 16.5% of the casein nitrogen could be accounted for in the wool grown, only 1.4% of the gelatin nitrogen could be so accounted for.

It was found in this experiment that less wool was grown from the 3.0 gm. cysteine per day supplement than from the 1.5 gm. per day supplement. The data are shown in Table (2.3).

Table (2.3). The Effect of Sulphur Containing Amino-acid Dosage on Wool Production. (from Reis and Schinkel, 1964).

Treatment	Wool grown due to Sa-a
60 gm. casein + 1.5 gm. cysteine	2.9 gm/day increase
casein + 3.0 gm. cysteine	1.7 gm/day increase
casein + 3.7 gm. methionine	2.8 gm/day increase
60 gm. gelatin + 1.5 gm. cysteine	2.3 gm/day increase
gelatin + 3.0 gm. cysteine	1.8 gm/day increase
gelatin + 3.7 gm. methionine	2.5 gm/day increase

This indicates a plateau response to increasing sulphur containing amino-acid dosages, a phenomenon also observed by Edwards (1954) with rats. In this experiment Edwards injected ^{35}S methionine intramuscularly and obtained a plateauing of label concentration in the hair as the dosage increased. Harper (1958) believed that this type of effect may be due to an amino-acid imbalance; possibly because other amino-acids become limiting.

Further work on this aspect of sulphur containing amino-acid administration has been done by Reis (1967). Small amounts (0.5 to 2.0 gm. per day) of cysteine or equimolar amounts of methionine increased the wool growth rate by up to 100% and the responses to both amino-acids were of similar magnitude. Wool production was reduced to slightly below the maximum response by 6.0 to 8.0 gm. cysteine per day, and to substantially below the

maximum response by equivalent amounts of methionine. In one case a heavy dose of methionine reduced wool production to below the pretreatment value. The proportion of administered sulphur accounted for in the extra wool grown was inversely related to the dose rate (23% to 49% at the 2.0 gm. dose level) but no dosage reduced the sulphur content of the wool. The responses to cysteine and methionine were similar at all dose rates, but no estimate could be made of an optimum dose rate.

Other effects of sulphur containing amino-acid administration have come to light. Work with rodents has shown the stimulatory effect of these amino-acids on liveweight gain, e.g. the work of Kwong and Barnes (1963) using iso-protein diets gave the results summarised in Table (2.4).

Table (2.4). Liveweight Gain Response to Sulphur Containing Amino-Acid Administration.

Treatment	Liveweight Gain/Day.
basal diet	4.1 gm.
+ 0.3% cystine	5.1 gm.
+ 0.3% methionine	5.0 gm.

Reis and Schinkel (1963; 1964) obtained small and larger increases respectively in the rate of liveweight gain following sulphur containing amino-acid administration, and Reis (1967) recorded small but consistent increases in body weight and found that the higher levels of administration did not retard liveweight

gain despite their effect on wool production.

Reis and Schinkel (1964) note that dry matter digestibility increased from 55% to 58% in all treatments involving sulphur containing amino-acids. They suggested that this may have been due to the recycling of nitrogen to the rumen or to an improved nitrogen / carbon ratio. Note also that Moir and Harris (1962) have obtained improved cellulose digestion in the rumen as a result of duodenal protein infusion.

The Effect of Sulphur Containing Amino-Acid Administration on the Character of the Wool Grown:

(a) the chemical composition and topography of wool fibre: Reis and Schinkel (1963) found that both cyst(e)ine and casein administration increased the sulphur content of wool by 24% to 35% and 15% to 20% of the basal content respectively. The efficiency of recovery of added sulphur in this experiment was high, 15% to 29% being recovered. In their (1964) experiment sulphur containing amino-acid administration increased wool sulphur content by 17% to 30% and casein increased it by 9% to 19%, the lower value being due to a greater dilution of sulphur in the greater amount of wool grown (Fraser, 1967). Again, and this has been a feature of all work done using the lower dose rates, the efficiency of incorporation of added sulphur was high.

When wool growth is inhibited to below the control level by high doses the sulphur content of the wool continues to rise (Reis, 1967). For example, when wool production was reduced by 24% following the infusion of 9.48 gm. methionine per day, the sulphur content of the wool grown rose from 3.22% to 3.76%.

Gillespie, et al. (1964) and Gillespie (1965) noted that an increase in sulphur content following sulphur containing amino-acid administration was due to an increase in the proportion and sulphur content of the high-sulphur proteins of the fibre, while there was possibly no change in the low-sulphur proteins. Gillespie, et al. (1964) noted that the ratio of high-sulphur to low-sulphur protein changed from 0.32 to 0.62 after cyst(e)ine administration, and also noted that cystine administration greatly increased the interfilamentous cement component of keratin.

The observations quoted here have largely borne out the predictions made in Part II of this Chapter on the effect on the chemical composition of the wool fibre of cyst(e)ine administration.

(b) fibre diameter: Marston (1935) and Reis and Schinkel (1960) have obtained increases in fibre diameter by administering sulphur containing amino-acids, and Schinkel (1962) increased fibre diameter by 31% by administering casein. Reis and Schinkel (1964) found that 30% to 40% of the observed increase in wool production was due to increased fibre diameter.

No data is available with respect to a differential effect on the diameter of the secondary and primary fibres or the S/P ratio, or on the proportion of medullated fibres. Smuts, et al. (1932) have, however, suggested that a dietary cystine supplement may reduce the medullation of rat hair.

(c) the physical properties of the fibre: (see Fraser, 1967): there is a reduced water uptake at relative humidity values

of above 80%. This may reflect a greater number of dithiol bonds but the reduction in water uptake is not as great as would be expected if all the added sulphur was in this form. That there is little difference between normal and sulphur rich wools in stress-extension behaviour supports the assumption that added sulphur is incorporated into the matrix. A greater torsional modulus in water is expected from the sulphur rich wool.

It is concluded that sulphur rich wools have no significant advantages or disadvantages compared with normal wools insofar as textile making is concerned, apart from increased fibre diameter.

CHAPTER 3. METHODS.

Introduction:

Two experiments were carried out: a preliminary comparison of the wool growth rates of sheep given intravenous infusions of cysteine at two different dose rates, and a study of the responses of sheep to cysteine infusion when fed two diets having different proportions of digestible crude protein. The effects of cysteine infusion on fibre diameter and the digestibility of the basal diet were also studied. A timetable of events over the course of the experiments is shown in Table (3.1).

Animals:

Twelve, three-year-old Romney wethers of varying wool types were shorn in November, 1966. They were subsequently placed in metabolism crates similar to that shown in Plate (3.1).

Digestibility Determinations:

As the animals were all of similar ages and past histories it was considered that six of them would give digestibility data applicable to the whole twelve. Animals numbers 1, 2, 3, 7, 11 and 12 were used in each experiment and were fed at the levels stated below in each trial.

The method described below was used to determine the DDM and DCP contents of the feeds investigated.

Table (3.1). Timetable of Events over the Experimental Periods.

No.	January	February	March	April	May	June	July	August	September
4			Dose Level Response Exp.		Dose Level Response Exp.		Re-allotment to two dietary protein levels	Protein Level Exp.	
5			Pre-experimental Wool Growth Period		Infusion Period	Post-experimental Period			
6	Control Wool Growth Period							Infusion Period	Post-exp. Period
8			High Protein Diet		High Protein Diet. Weekly Wool Samples	High Protein Diet. Weekly Wool Samples			
9									
10			18 Feb to 5 May		15 May to 15 June	15 June to 19 July			
	High Protein Diet							Wool Samples on 17 Aug. and 7 Sept.	Wool Samples on 28 Sept
1									
2			Digestibility Experiment		Digestibility Experiment				
3				Re-allotment to Low Protein Diet					
7	Nov 1966 to 18 Feb 1967								
11			High Protein Diet		Low Protein Diet			2 Aug to 27 Sept	7 Sept to 28 Sept
12									

(a) collection: the trials lasted for twelve days, after a preliminary period to allow the animals to accustom themselves to the new feed. They were fed 0.45 Kg. in the morning and 0.68 Kg. at night and refusals were collected before the next feeding and weighed. The weight fed minus the weight refused was taken as the weight of feed eaten, there being only a slight loss of feed on to the floor and no appreciable difference in dry matter content between the feed fed and that refused.

Faeces collection started on the third day. The animals were standing on a wire grating and the faeces were collected in a plastic bucket. No attempt was made to stop aerobic fermentation as this was considered to be negligible and the slight urine contamination of the faeces was also considered to have a negligible effect on the faecal nitrogen content. Some pellets lodged between the grating and the frame of the pen and although most of these were scraped into the bucket at each collection there was some inevitable loss. The use of plastic strips, in the third trial, to line the edges of the grating partly solved this problem. Three animals were fitted with faeces bags in the second experiment but the average digestibility figure obtained from these animals was not different from that obtained from those animals whose faeces were collected in buckets.

The faeces were weighed wet and then frozen until the crude protein analyses were made. This method reduced the loss of faecal nitrogen to a minimum but may have allowed the loss of small quantities of water.

(b) nitrogen analyses:

(i) of the feed: samples of the feed were taken at random from the bin. These were ground and bulked, and a Kjeldahl analysis was done in triplicate on approximately 1.0 gm. subsamples.

(ii) of the faeces: the total faecal output of each sheep was bulked and approximately one quarter of this was taken and mixed. A second subsample of approximately 150 gm. weight was taken and macerated in about 400 ml. of distilled water. About 3.0 to 4.0 gm. of the macerate was used for each Kjeldahl analysis. Each analysis was done in triplicate.

(c) dry matter determinations: the dry matter contents of the feeds, faeces and faeces macerates were determined by drying duplicate samples in a hot-air oven for not less than 48 hours at about 95°C.

Feeding Regimes:

The feed used in the dose level response experiment and as the high protein diet in the protein level experiment was milled ryegrass-clover hay having a digestible dry matter (DDM) content of 53.7% and digestible crude protein (DCP) content of 9.9%. The sheep receiving this feed were given 1.1 Kg. (2.5 lbs.) per day (i.e. a level slightly above that calculated to maintain body weight). In practice no animal gained in weight, a stable liveweight being reached within three weeks.

The low protein feed used in the protein level experiment was a mixture of 1.5 parts of milled ryegrass-clover hay to 1.0 parts of chaffed barley straw. This composite feed had a DDM content of 39.7% and a DCP content of 7.0%. This feed was not very palatable and the animals fed it would not eat more than about one kilogram per day. Consequently the sheep under the low protein regime were also given 1.1 Kg. of feed per day. Their DDM intakes were about 0.16 Kg. (or about 23%) lower than those of the sheep under the high protein regime; and their DCP intakes were about 0.032 Kg. per day (or about 41%) lower.

Water was given ad lib.

All feeding regimes were continued until the end of the post experimental periods.

Cysteine Experiments:

The sheep were infused with a sterile solution of either physiological saline or of L-cysteine hydrochloride made up into an isotonic solution with saline. The solutions were infused via a polythene catheter inserted into the jugular vein (see Plate (3.2). The apparatus used is shown in Plates (3.1) and (3.3).

(a) dose level response experiment: this experiment involved sheep numbers 4, 5, 6, 8, 9 and 10; two of which were allotted at random to each treatment.

There were three treatments:

(i) control: 500 ml. saline per day.

Plate (3.1). A Metabolism Crate Showing the Collection and Infusion Apparatus.

Plate (3.2). The Catheter in Position in the Jugular Vein.

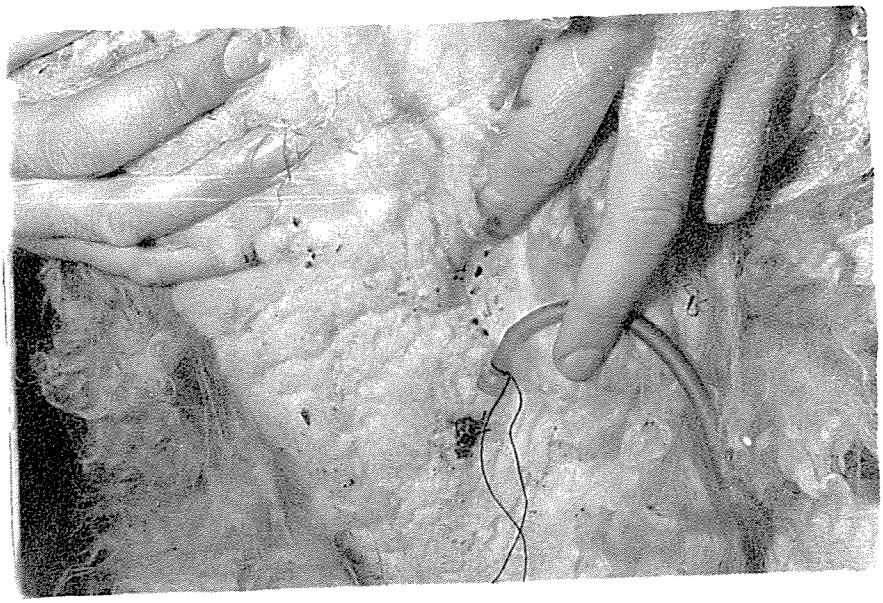
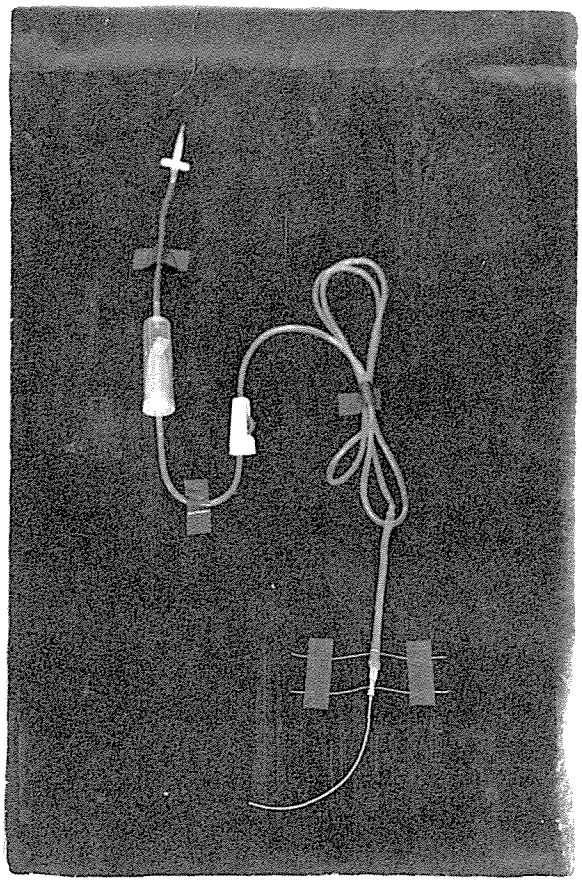


Plate (3.3). The 'Tuta' Infusion Apparatus with the Catheter Attached.



(ii) 2 gm.: 2.0 gm. cysteine made up to 500 ml. of isotonic solution with saline per day.

(iii) 4 gm.: 4.0 gm. cysteine made up to 500 ml. of isotonic solution with saline per day.

The infusion continued for five weeks.

Table (3.2). Allottment of Treatments in the Protein Level Experiment.

No.	Previous Treatment	New Treatment
1	low protein, no infusion	low protein, cysteine
2	low protein, no infusion	high protein, cysteine
3	low protein, no infusion	high protein, cysteine
4	high protein, cysteine	low protein, saline
5	high protein, cysteine	high protein, cysteine
6	high protein, saline	low protein, cysteine
7	low protein, no infusion	low protein, saline
8	high protein, cysteine	low protein, cysteine
9	high protein, cysteine	high protein, saline
10	high protein, saline	high protein, saline
11	low protein, no infusion	low protein, saline
12	low protein, no infusion	high protein, saline

Note: No. 5 was withdrawn from the experiment on 13 August due to difficulty with the catheter.
No. 6 was withdrawn from the experiment on 6 August and used as a low protein control.
No. 7 was changed to low cysteine on 10 August to replace No. 6.

(b) protein level experiment: all the sheep were involved and were allotted to the treatments in the manner shown in Table (3.2). Two weeks were allowed for their wool growth rates and liveweights become equilibrated at the new levels of feeding.

The Treatments were:

(i) low protein - cysteine (LC).

(ii) low protein - saline (LS).

(iii) high protein - cysteine (HC).

(iv) high protein - saline (HS).

Three animals were allotted to each treatment. The sheep receiving saline acted as controls and received 500 ml. of saline per day. The cysteine treated sheep were infused with 2.0 gm. cysteine made up to 500 ml. of isotonic solution with saline per day.

The infusion continued for five weeks.

Wool Sampling and Measurement Methods:

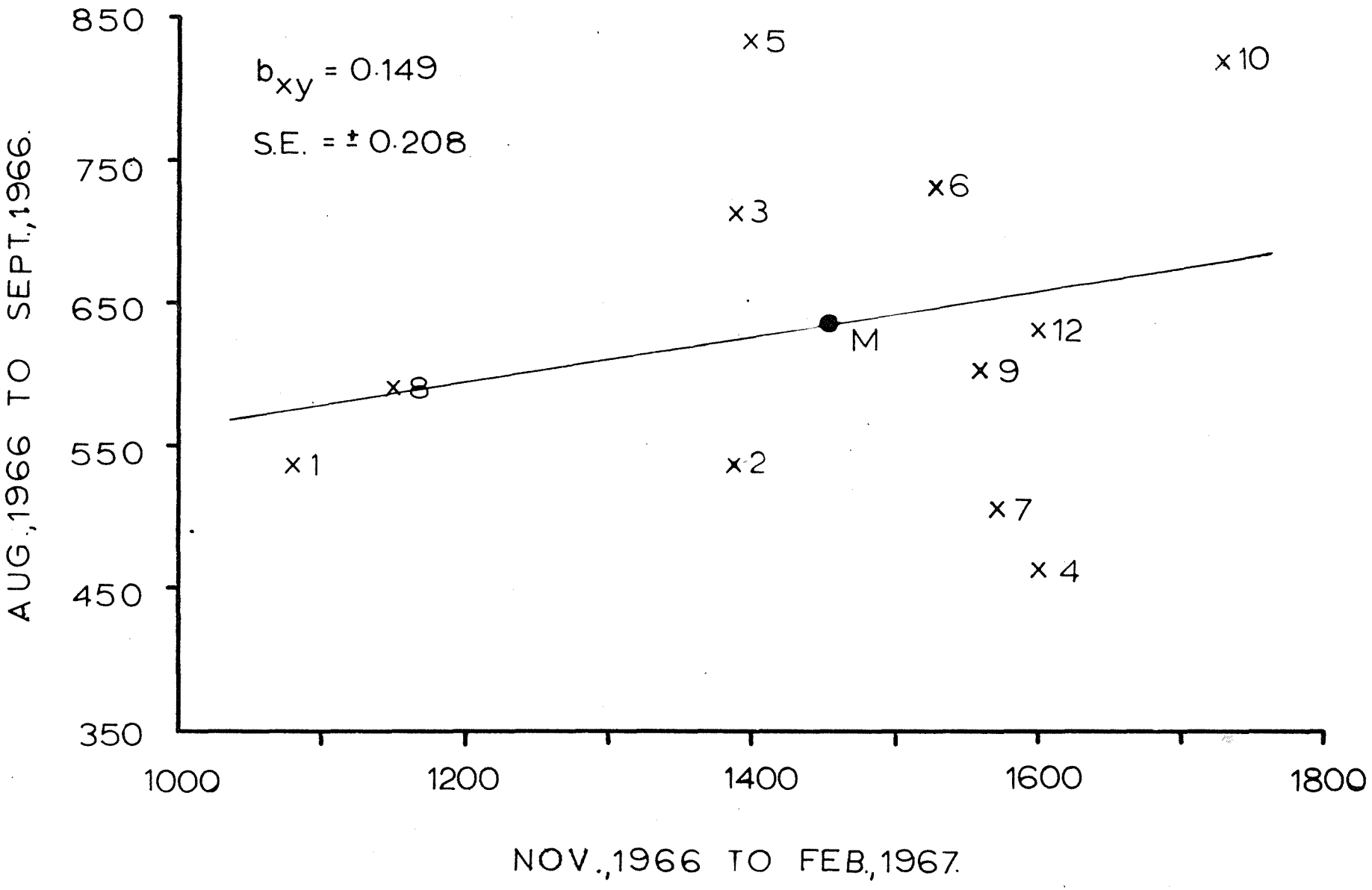
Wool samples were removed from the sheep using the fine blade (0000) of 'Oster' small animal clippers.

During the control periods wool samples were taken from a

patch of about 100 cm^2 on the right mid-side of the sheep. The control period used in the dose level response experiment was from 18 February to 5 May. The pre-experimental control wool growth rates used in the protein level experiment were obtained from averaging the growth rate values obtained during August to September, 1966, and November, 1966 to February, 1967. These values, when plotted against each other gave the relationship shown in Fig. (3.1). These control wool growth values effected a good separation of the various treatment groups as is shown in Fig. (3.2), and accounted for approximately 70% of the within subclasses sum of squares of the experimental data. (see Table 4.6).

For the dose level response experiment three approximately 50 cm^2 patches were delineated on the sheeps mid-side: two on the right side and adjacent to each other and the third on the left. The patches were clipped at the beginning of the experiment and then sampled in rotation at weekly intervals so that one weeks growth was taken from the first patch, two weeks from the second patch and three weeks from the third. The four-week sample weight was obtained by sampling the first patch at the fourth week and adding the one-week sample weight already taken from that area. The fifth week sampling was made twice from the second and third patches in a similar way. The rotation is depicted in Fig. (3.3).

THE PROTEIN LEVEL CONTROL WOOL GROWTH RATE VALUES.



VALUES USED.

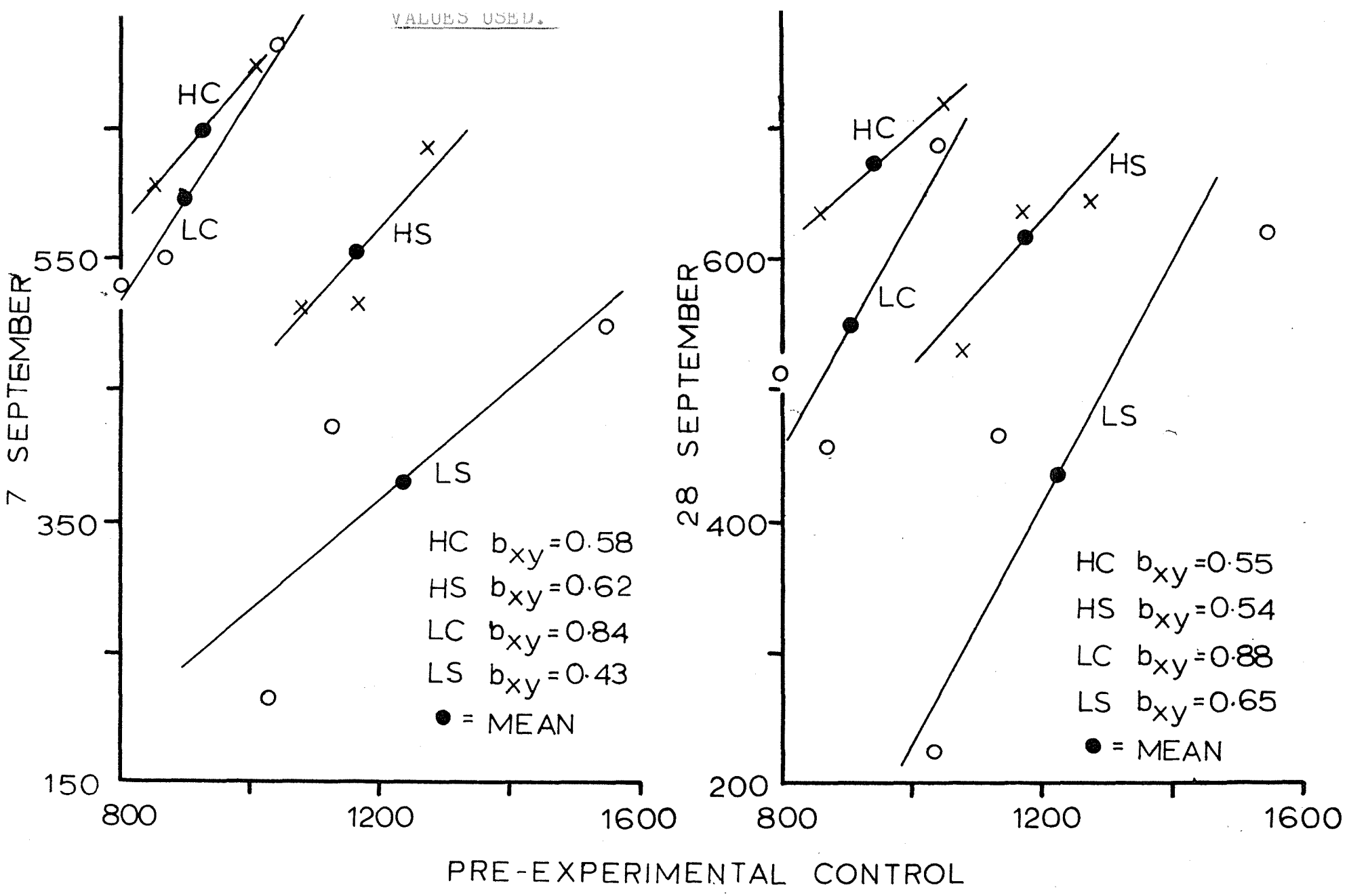
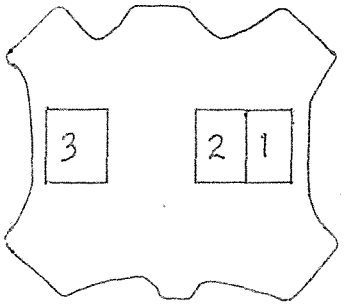

HC
LC
HS
LS
550
350
150
800
1200
1600
7 SEPTEMBER
HC b_{xy}=0·58
HS b_{xy}=0·62
LC b_{xy}=0·84
LS b_{xy}=0·43
● = MEAN
600
400
200
28 SEPTEMBER
HC b_{xy}=0·55
HS b_{xy}=0·54
LC b_{xy}=0·88
LS b_{xy}=0·65
● = MEAN
PRE-EXPERIMENTAL CONTROL

Fig. (3.3). Sampling Rotation for the Dose Level Response Experiment.



Only the right side patches were sampled during the post-experimental period. The sampling rotation is shown in (Table (3.3).

Table (3.3). Sampling Rotation During the Dose Level Response Experiment Post-experimental Period.

Patch	Sampled At
1	1, 3, 4, 5 weeks
2	2, 4, 5 weeks

For the protein level experiment two approximately 100 cm^2 patches were delineated, one on the right mid-side and one on the left, and approximately 50 cm^2 shoulder and rump patches were delineated. These patches were clipped at the beginning of the experiment and then sampled in the manner shown in Table (3.4).

Table (3.4). Sampling Rotation for the Protein Level Experiment.

Patch	Sampled At
Right Mid-side	2, 5 weeks
Left mid-side	5 weeks
Shoulder	5 weeks
Rump	5 weeks

All patches were sampled three weeks after the end of the infusion period.

The wool sampled was conditioned at about 65% relative humidity and 18°C for not less than 20 hours and then weighed. The samples were scoured by soaking them in two baths of petroleum ether and a final bath of hot water, each soaking lasting for about four minutes. The cleansed samples were then conditioned and weighed.

The mean fibre diameters of the scoured samples were measured using the projection microscope.

Method of Data Analysis:

All wool weight data was expressed as mg. x 10^{-3} wool per cm^2 per day.

(a) dose level response experiment:

(i) analysis of variance:

model: $x_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$

where: x_{ijk} = jk^{th} observation in the i^{th} A class.

μ = overall mean.

a_i = effect of the i^{th} Treatment class.

b_j = effect of the j^{th} Sides class.

$(ab)_{ij}$ = individual interaction effects.

e_{ijk} = random error.

and where: e_{ijk} is normally distributed with a mean of zero and a standard deviation of σ, i.e. $e_{ijk} = N(0,\sigma)$.

The components of the various sums of squares are shown below. (Note: for 'R' read 'fitting').

$$\text{Total} = R(\mu\ a_i\ b_j\ (ab)_{ij}\ e_{ijk}).$$

$$\text{Between Subclasses} = R(\mu\ a_i\ b_i\ (ab)_{ij}).$$

$$\text{Interaction} = R(\mu\ a_i\ b_i\ (ab)_{ij}) - R(\mu\ a_i\ b_j).$$

The computations are shown below (Cockrem, pers. comm.):

$$\text{Interaction SS} = \left[\text{B. Subclass}\right] - \left[(\text{B. Treatments}) + (\text{B. Sides})\right].$$

All other computations are as for an orthogonal analysis.

(ii) analysis of covariance:

Model: $x_{ij} = \mu + a_i + \beta x_{ij} + e_{ij}$

where: βx_{ij} = slope of the regression line fitting the population comprised of the terms x_{ij}

and where: $e_{ij} = N(0,\sigma)$.

The computational methods are those of Snedecor (1956) for covariance in a completely randomised experiment with two treatments.

(b) <u>protein level experiment</u>: a 2 x 2 factorial design with disproportionate subclass numbers owing to the withdrawal of sheep number 5.

(i) analysis of variance:

model: $x_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$

where: x_{ijk} = k^{th} observation in the j^{th} B class and the i^{th} A class.

μ = overall mean with equal subclass numbers.

a_i = effect of the i^{th} Diets class.

b_j = effect of the j^{th} Treatment class.

$(ab)_{ij}$ = individual interaction effects expressed as deviations from μ.

and where: e_{ijk} = $N(0,\sigma)$.

The components of the sums of squares are:

Total = $R(\mu\ a_i\ b_j\ (ab)_{ij}\ e_{ijk})$.

B. Subclass (with interaction) = $R(\mu\ a_i\ b_j\ (ab)_{ij})$.

B. Subclass (without interaction) = $R(\mu\ a_i\ b_j)$.

B. Class = $R(\mu\ a_i\ b_j\ (ab)_{ij}) - R(\mu\ b_j\ (ab)_{ij})$.

= a_i

Interaction = $R(\mu\ a_i\ b_j\ (ab)_{ij}) - R(\mu\ a_i\ b_i)$.

= $(ab)_{ij}$.

The constants and sums of squares with interaction were obtained by a weighted means analysis following Harvey (1960). Those for the $R(\mu\ a_i\ b_j)$ were computed from algebraic formulae obtained from the least squares equations which lead to a simplified form for the 2 x 2 factorial case (Cockrem, pers. comm.).

e.g. B. Class S.S. (with interaction) for A

$$= \frac{v_1 v_2}{v_1 + v_2} (\bar{A}_1 - \bar{A}_2)^2$$

Where $\frac{v_1 v_2}{v_1 + v_2}$ = weighting factor calculated from the numbers in each subclass.

$$\bar{A}_1 = \frac{(\bar{x}_{11} + \bar{x}_{22})}{2}$$

$$\bar{A}_2 = \frac{(\bar{x}_{12} + \bar{x}_{22})}{2}$$

$$R(\mu\ a_i\ b_j) = (\mu\hat{} + b_i)x_{1.} + (\mu\hat{} + b_2)x_{2.} + (\hat{a_1})\ x_{.1} + (\hat{a_2})x_{.2}$$

where:

$$(\hat{a_1}) = \left[(\bar{x}_{11} - \bar{x}_{12})/z_1 + (\bar{x}_{21} - \bar{x}_{22})/z_2\right] /2u.$$

$$(\hat{a_2}) = -(\hat{a_1})$$

$$(\mu\hat{} + b_1) = \bar{x}_{1.} - v(\hat{a_1}).$$

and where: μ, v, w, z_1, z_2 = weighting factors calculated from the numbers in each subclass.

x = total of individual items of data specified. (see Table 3.5 for notation).

The remaining computations are those used in orthogonal analyses.

Table (3.5). Notation Used in Computations for Analysis of Variance Sums of Squares.

	A_1	A_2	Sum
B_1	x_{11}	x_{12}	$x_{1.}$
B_2	x_{21}	x_{22}	$x_{2.}$
Sum	$x_{.1}$	$x_{.2}$	

(ii) analysis of covariance:

model: $x_{ijk} = \mu + a_i + b_j + (ab)_{ij} + \beta x_{ijk} + e_{ijk}$

where: $e_{ijk} = N(0,\sigma)$.

The components of sums of the sums of squares are:

$$\text{Total} = R(\mu\ a_i\ b_j\ (ab)_{ij} \beta\ x_{ijk}\ e_{ijk}).$$

$$\text{B. Subclass} = R(\mu\ a_i\ b_j\ (ab)_{ij}).$$

$$\text{W. Subclass} = \left[\text{Total}\right] - \left[\text{B. Subclass}\right].$$

$$= \beta x_{ijk} + e_{ijk}$$

$$\text{Interaction} = \left[(\text{Total}) - R(\mu\ a_i\ b_i)\right] - \left[\text{W. Subclass}\right].$$

$$= \left[(ab)_{ij} + \beta x_{ijk} + e_{ijk}\right] - \left[\beta x_{ijk} + e_{ijk}\right]$$

$$= (ab)_{ij}.$$

When the Interaction is not significant, the B. Diets, W. Diets, and Total terms are obtained as for an orthogonal analysis

and the sums of squares for testing B. Classes are obtained as follows:

$$\text{e.g. Between A S.S.} = R(\mu\ a_i\ b_j \beta x_{ijk}) - R(\mu\ b_j \beta x_{ijk})$$

$$= \left[(\text{Total}) - R(\mu\ a_i\ b_j \beta x_{ijk})\right] - \left[(\text{Total}) - R(\mu\ b_j \beta x_{ijk})\right]$$

$$= \left[\text{Residual S.S. fitting regression within Treatments}\right] - \left[\text{Residual S.S. fitting regression within Treatments and within Diets}\right].$$

The computational methods are those described for the analysis of variance.

CHAPTER 4. RESULTS.

Dose Level Response Experiment:

It was found that the method of obtaining the sample weights described in Chapter 3 did not give satisfactory results for these reasons:

(i) the low winter wool growth rate led to small sample sizes.

(ii) the animals weekly wool growth rates showed a large variation even when the samplings were done on the same area.

(iii) it was considered that the anatomical differences (e.g. rumen distension) between the right and left side sample patches would preclude a meaningful comparison of wool growth rates between these areas, and it was also found that there were differences in estimates of wool growth rate between adjacent areas on the same side.

(iv) it was considered that any errors made in sampling the two adjacent right side patches could be confounded, i.e. an over-estimate of one patch leading to a corresponding underestimate of the other.

The magnitude of these errors was such as to lead to a minus value for wool growth in one case.

In view of these considerations is was decided to use the control period sample weights obtained as described in Chapter 3 and the sample weights taken from patches 2 and 3, and to express these results as mean daily wool growth per unit area.

(a) wool growth rate response: the results obtained are shown in Table (4.1) and Fig. (4.1).

It can be seen from Fig. (4.1) that the treatment effects became most noticeable at the end of the infusion period (15 June) and that the response to cystine infusion continued during the immediate post-experimental period (until 28 June) after which it decreased until by 19 July (five weeks after the end of the infusion period) no residual response was observable.

Analyses of variance and covariance were made on the data obtained from the 15 June sampling, see Table (4.2) and (4.3).

No pre-experimental control data was available for the left mid-side patch, so only the right mid-side data could be used. No extra precision was obtained in the covariance analysis, because of this, although the pre-experimental control data did remove about 60% of the variation in the Within Treatment sum of squares of 'y'. An extension of this analysis was made, similar to that of the analysis of variance, but in this case

Table (4.1). Mean Daily Wool Growth from Patch 2 ($mg.x10^{-3}/cm^2/day$).

No.	Treatment	Pre-expt. Controls	Experimental			Post-Experimental		
			24 May	15 June	14 June (Patch 3)	28 June	12 June	19 July
4	2 gm.	955	574	533	461	474	275	473
9	2 gm.	878	635	801	1112	848	157	447
Mean 2 gm.		916.5	604.5	667		661	216	460
5	4 gm.	675	571	624	618	594	238	483
8	4 gm.	731	536	599	634	704	283	483
Mean 4 gm.		703	553.5	611.5		649	260.5	483
Mean cysteine		809.75	579	639.25		655	238.25	471.5
6	saline	869	667	489	538	259	215	424
10	saline	1001	489	380	271	460	242	445
Mean saline		935	578	434.5		359.5	228.5	434.5

Table (4.2). Analysis of Variance of Data from 15 June Sampling.

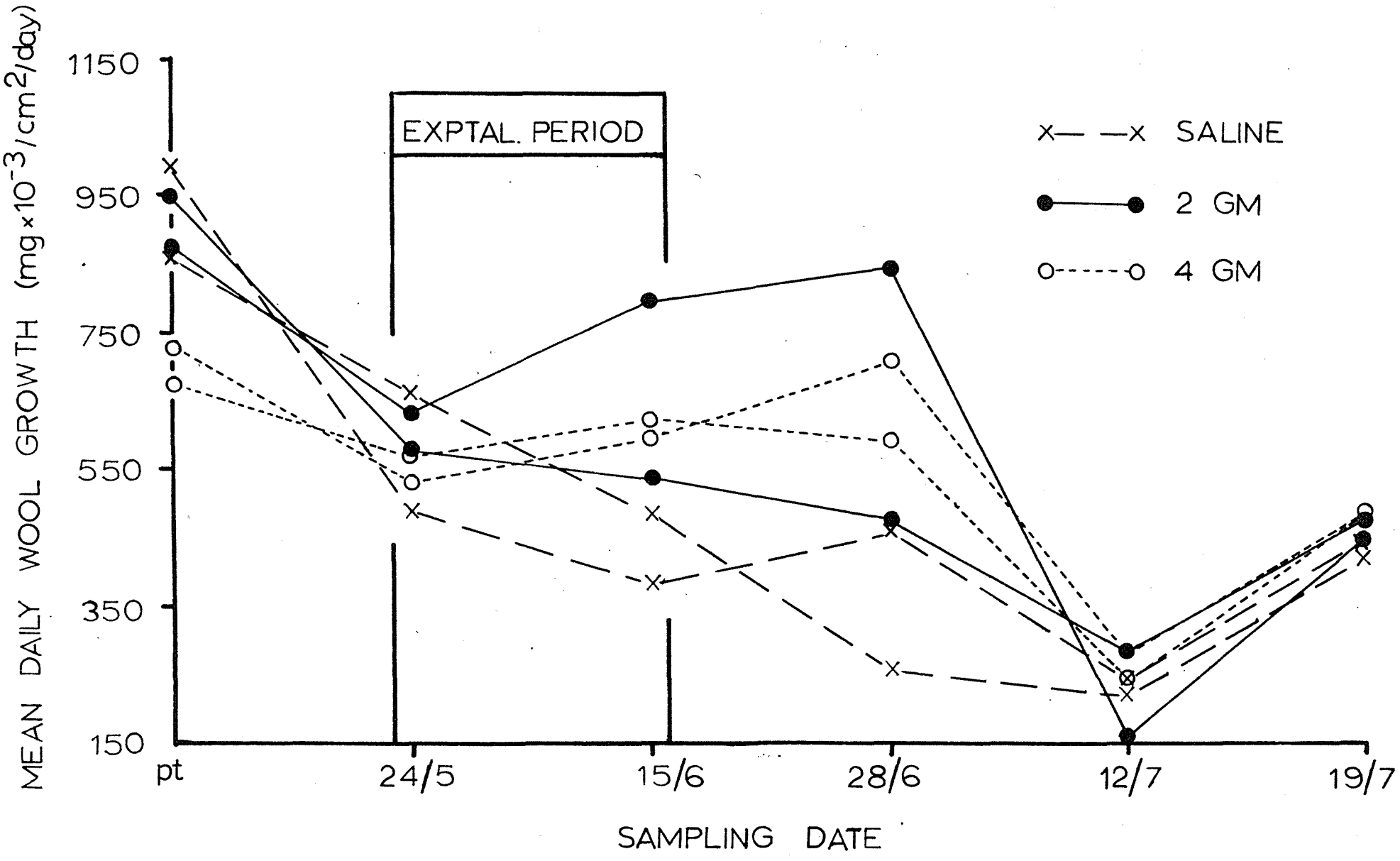
Source	SS	df	ms	F
Total	499584.6667	11	45416.7879	
B. Treats.	194356.1667	2	97178.0833	2.01 N.S.
B. Sides.	3605.3267	1	3605.3267	0.07 N.S.
Interact.	11785.1733	2	5892.5867	0.12 N.S.
W. Subclass.	289838.0000	6	48306.3333	
B. Cyst.v Sal.	171028.1667	1	171028.1667	4.74 * (p=0.06)
B. Sides.	3605.3267	1	3605.3267	0.09 N.S.
Error	324951.1733	9	36105.6859	

Table (4.3). Analysis of Covariance of Data from 15 June Sampling.

(x = pre-experimental control, y = experimental)

Source	df	x^2	xy	y^2	b_{xy}	df	$\sum d$	F
2 gm.	1	2964.5	-10318	35912.0				
4 gm.	1	1568.0	-700	312.5				
Saline	1	8712.0	-7194	5940.5				
W. Treats.	3	13244.5	-18212	42165.0	-1.3751	2	17121.6788	
Total	5	79743.5	-40556	101142.0	-0.5086	4	80515.2184	
B. Treats.						2	63393.5396	3.7 N.S.

FIG. (4.1). WOOL GROWTH RATE RESPONSES TO CYSTEINE INFUSION.



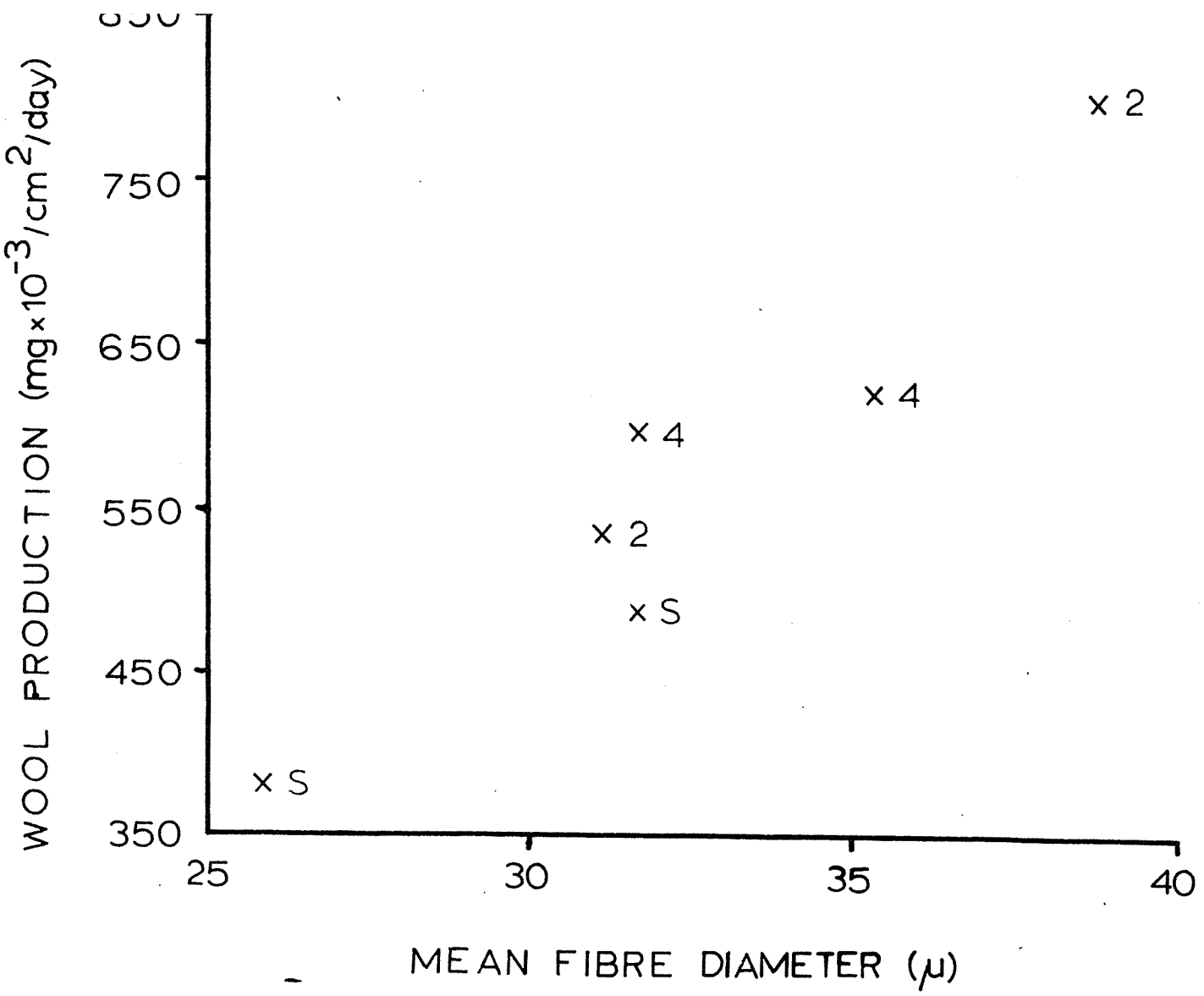
the pre-experimental control data removed almost none of the variation.

Fig. (4.1) shows that the pattern of response at 28 June is very similar to that at 15 June so no further statistical analysis was undertaken.

There was a significantly different wool growth rate response between the cysteine treated animals and the control animals at the dietary crude protein level fed and at this stage of the wool growth cycle. The sheep receiving 2.0 gm. cysteine per day showed a mean increase of 53.51% in wool grown per $cm.^2$. per day over the controls at the 15 June sampling, while those receiving 4.0 gm. per day showed an increase of 40.74%. The cysteine treated animals showed a mean increase of 47.12% over the controls at this sampling. For the 28 June sampling the increases were 83.87%, 80.53% and 82.19% respectively.

It was concluded that, in the conditions of diet and seasonal wool growth described, cysteine infusion does increase the wool growth rate of the New Zealand Romney, and that the response to the infusion of 2.0 gm. cysteine per day is slightly greater than that due to the infusion of 4.0 gm. per day.

(b) <u>fibre diameter response</u>: the mean fibre diameters of the samples taken from patch 2 on 15 June and plotted against the corresponding mean daily wool growth figures are shown in Fig. (4.2). The sample standard deviations of fibre diameter for the


WOOL PRODUCTION (mg×10⁻³/cm²/day)
750
650
550
450
350
25
30
35
40
MEAN FIBRE DIAMETER (μ)
x 2
x 4
x 4
x 2
x S
x S

whole experiment were averaged over each treatment and are shown plotted with their respective fibre diameter treatment means in Fig. (4.3).

The mean fibre diameters and their corresponding wool growth rates are positively correlated, indicating that an increase in fibre diameter contributed to the increase in wool production obtained.

The means and standard deviations follow much the same trends, indicating that all fibres were probably affected to the same extent by the cysteine treatments. However, the saline treated animals standard deviations increased, while their fibre diameter means descreased. This was probably due to the finer fibres decreasing in diameter to a greater extent than the coarser ones.

A more detailed examination of the fibre diameter - wool growth rate relationship was made in the following experiment.

Protein Level Experiment:

(a) wool growth rate response: the wool growth rate responses obtained from the four treatments are shown in Table (4.4) and Fig. (4.4).

Again, as is shown in Fig. (4.4), the cysteine treatment effects became most noticeable towards the end of the infusion period and continued during the three-week post-experimental period.

Table (4.4). Mean Daily Wool Growth from Right Mid-Side Patch. (mg. x 10^{-3} / cm^2 / day).

No.	Treat.	Pre-exptal. Control	Experimental		Post-exptal
			17 Aug.	7 Sept.	28 Sept
2	HC	863	678	605	633
3	HC	1051	716	694	717
Mean HC		957	697	649.5	675
9	HS	1082	669	516	528
10	HS	1281	715	634	643
12	HS	1165	585	518	634
Mean HS		1176	656.3	556	601.6
1	LC	808	521	530	512
7	LC	1037	567	714	686
8	LC	870	543	547	456
Mean LC		905	543.6	597	551.3
4	LS	1032	352	214	224
6	LS	1129	580	423	463
11	LS	1550	617	498	622
Mean LS		1237.0	516.3	378.3	436.3
Mean High Protein		1088.4	672.6	593.4	631.0
Mean Low Protein		1071.0	530.0	487.6	493.8
Mean Cysteine		925.8	605.0	618.0	600.8
Mean Saline		1206.5	586.3	467.2	519.0

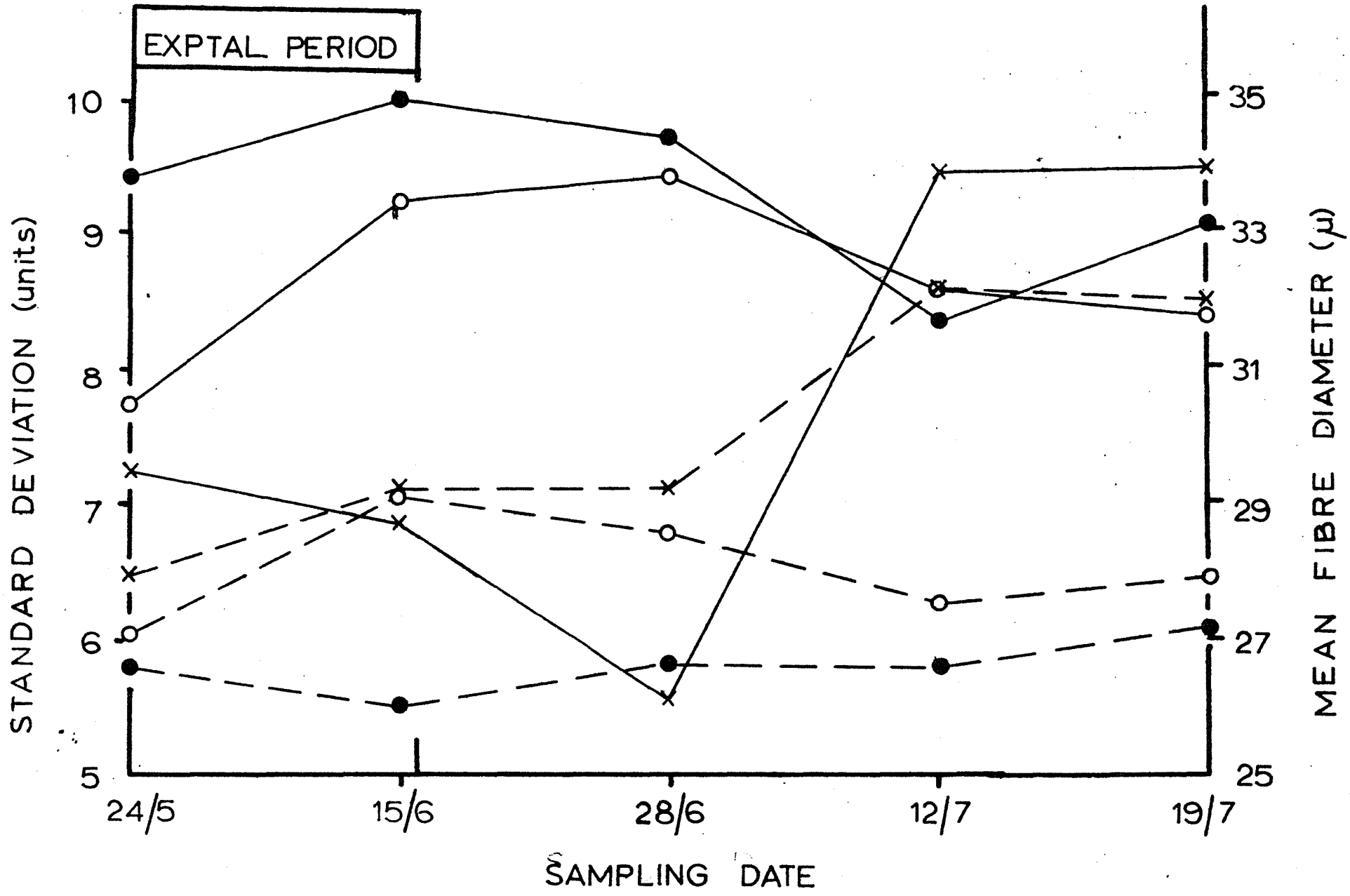
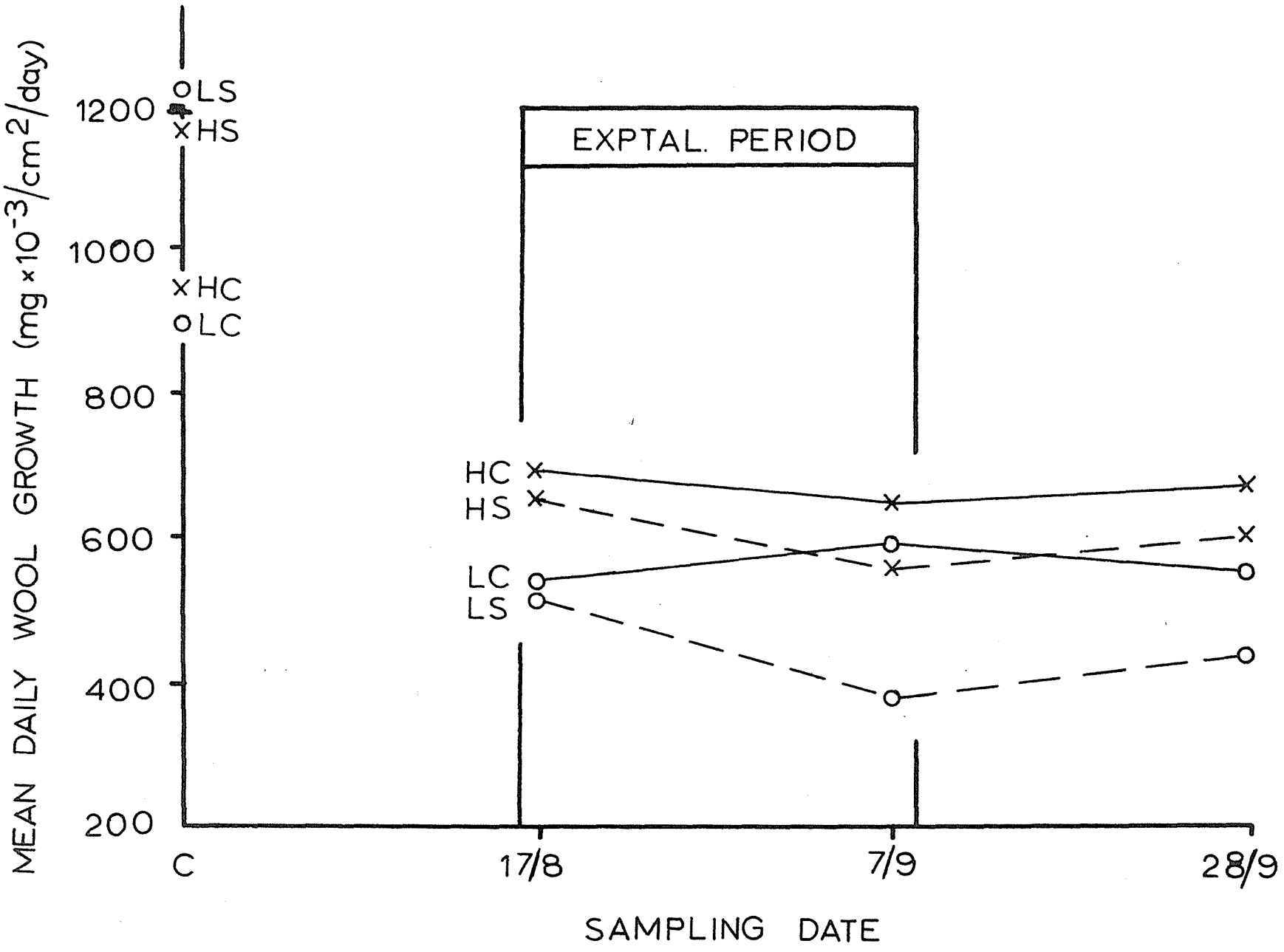

EXPTAL PERIOD
STANDARD DEVIATION (units)
MEAN FIBRE DIAMETER (μ)
10
9
8
7
6
5
35
33
31
29
27
25
24/5
15/6
28/6
12/7
19/7
SAMPLING DATE

FIG. (4.4.). WOOL GROWTH RATE RESPONSES OBTAINED IN THE PROTEIN LEVEL EXPERIMENT.



An analysis of variance on the data obtained at the 7 Sept. sampling (Table 4.5) indicated that differences existed between the treatment groups, but differences between animals in the pre-experimental control period suggested the value of a covariance analysis (Table 4.6). An interaction between the diet and treatment effects was found so the subclasses were tested for difference (Table 4.7).

Table (4.8) shows the analysis of covariance made on the data from the 28 Sept. sampling.

As the interaction term was not significant the full covariance analysis without interaction was completed.

As the between subclasses terms in the analyses of both samplings were found to be significantly different, the subclass means were adjusted to the mean of the pre-experimental control wool growth rate values and a multiple range test (Duncan, 1955) used to show how the adjusted means differed from each other. This data is shown in Table (4.9). The range tests involved a slight under-estimate for the HC subclass which had only two members.

The adjusted means show that cysteine treatment increased the mean wool growth rate of the animals fed the high protein diet by 40.49% over the control animals mean value at the 7 Sept. sampling, and by 40.31% at the 28 Sept. sampling. The wool growth rates of the treated animals on the low protein diet were

Table (4.5). Analysis of Variance of Data Taken at the End of the Infusion Period (7 Sept.).

Source	SS	df	ms	F
Total	189790.182	10	189790.0182	
B. Diets.	35318.2172	1	35318.2172	3.21 N.S.
B. Treats.	64966.1361	1	64966.1351	5.9 *
W. Subclass.	77087.167	7	11012.452	
Interact.	10136.9354	1	10136.9354	0.92 N.S.

Table (4.6). Analysis of Covariance of Data Taken at the End of the Infusion Period.

(x = pre-experimental control, y = experimental).

Source	df	x^2	xy	y^2	b_{xy}	df	$(b_{xy})(xy)$	$\sum d$
Total	10	441084.91	-17703.272	189790.182	-0.04104	9	726.5423	189079.5727
B. Subclass	3	223714.91	-128450.272	112703.015	-0.1271	2	3614.8433	109098.1717
W. Subclass	7	217370.00	110747.000	77087.167	0.5095	6	56425.5965	20661.5705
μab	2	215202.2609	-119020.1386	102566.0896	-0.5531	1	65830.0387	36427.2769
Total - μab	8	225882.6491	101316.8666	87224.0924	0.4485	7	45440.6147	41783.4777

To test Interaction:

(Total - μab Res.) - (W. Subclass Res.) = 21121.9072

Interaction m.s. = 21121.9072

W. Subclass m.s. = 3443.5951

F (1,6) = 6.13 *

To test W. Subclass. Regression:

$(b_{xy})(xy)$ = 56425.5965

Regression m.s. = 56425.5965

W. Subclass m.s. = 3443.5951

F (1,6) = 16.39 **

Note: this test justifies the use of the pre-experimental control period selected. It accounts for about 70% of the Within Subclasses sum of squares of y. See also Table (4.8).

Table (4.7). Test of Difference Between Subclasses.

(Total Res) - (W. Subclass Res)	=	168418.0022
B. Subclass. m.s.	=	56319.3340
W. Subclass. m.s.	=	3443.5951
F(3,6)	=	16.3025***

Table (4.8). Analysis of Covariance: Data Taken at the End of the Post-experimental Period (28 Sept.). (x = pre-experimental control, y = experimental).

Source	df	x^2	xy	y^2	b_{xy}	df	$(b_{xy})(xy)$	Σ d
Total	10	441084.91	72462.182	198351.637	0.1643	9	11905.5365	186446.1005
B. Subclass.	3	223714.91	-70032.818	77603.6369	-0.3130	2	21920.2720	55683.3649
W. Subclass.	7	217370.00	142495.000	120748.0001	0.6555	6	93405.4725	27342.5276
μab	2	215202.2609	-66892.8472	76444.8782	-0.3108	1	20790.2969	55654.5813
Total - μab	8	225882.6491	139355.0292	121906.7588	0.6169	7	85968.1175	35938.6413

To test Interaction:

(Total - μab Res.) - (W. Subclass. res.) = 8596.1137

Interaction m.s. = 8596.1137

W. Subclass. m.s. = 4557.0879

F(1,6) = 1.88 N.S.

To test W. Subclass. Regression:

$(b_{xy})(xy)$ =93405.4725

Regression m.s. =93405.4725

W. Subclass. m.s. = 4557.0879

F(1,6) = 20.49**

continued:

Table (4.8). Continued.

Source	df	x^2	xy	y^2	b_{xy}	df	$(b_{xy})(xy)$	Σd
B. Diets.	1	825.71	6509.182	51312.803	7.8831			
W. Diets.	9	440259.20	65953.000	147038.834	0.1498	8	9879.7594	137159.0746
B. Treats.	1	214888.61	-62621.618	18248.837	-0.2914			
W. Treats.	9	226196.30	135083.800	180102.800	0.5972	8	107557.3922	72545.4078

To test Diets: (W. Treats. Res.) - (Total - μab Res.) = 36606.7665

B. Diet m.s. = 36606.7665

W. Subclass. m.s. = 4557.0879

F(1,6) = 8.03*

To test Treatments: (W. Diet. Res.) - (Total - μab Res.) = 101220.4333

B. Treat. m.s. = 101220.4333

F(1,6) = 22.21***

Table (4.9). Adjusted Means and Multiple Range Tests.

ADJUSTED MEANS

7 Sept.

Exptal. Means.

	H	L
C	649.5	597
S	556	378.3333

Adj, Means.

	H	L
C	711.6127	685.6067
S	506.5322	297.7860

28 Sept.

	H	L
C	675	551.3333
S	601.6667	436.3333

	H	L
C	754.9114	658.3307
S	538.0236	332.7047

Continued:

Table (4.9). Continued.

RANGE TEST ON ADJUSTED MEANS

7 Sept. S.E. = 38.86

Comparison	2	3	4
p	5.24	5.51	5.65
Range	117.43	186.57	191.31

Treats.	LS	HS	LC	HC
Means.	297.79	506.53	685.61	711.61

28 Sept. S.E. = 38.97

Comparison	2	3	4
p	5.24	5.51	5.65
Range	204.20	214.72	220.18

Treats.	LS	HS	LC	HC
Means.	332.70	538.02	665.33	754.91

Note: Underlined means are not significantly different at the 1% level.

increased by 130.23% and 99.98% at the two samplings. This comparison illustrates the nature of the interaction found in the analysis of the 7 Sept. data.

The two diets were found to cause significant differences in wool growth rate but this difference was less significant than that caused by the cysteine treatment. The mean wool growth rate of the animals fed the high protein diet was increased by 70.09% above that of the low protein fed animals at the 7 Sept. sampling, and by 61.71% at the 28 Sept. sampling. These mean differences were calculated from the saline control data, rather than the whole, in order to obtain an estimate unbiased by the cysteine treatment×diet interaction.

The effect of the treatments on the wool growth rate gradient over the sheep is shown in Table (4.10) where the ranked subclass means for each sample patch is shown.

The contradictory results given by the left mid-side patches are probably due to the unreliability of this area for wool growth rate measurement purposes. The ranking of the shoulder and rump subclass means for the 7 Sept. sampling is not the same as that for the mid-side patch subclass means, indicating a possible patch x treatment interaction during the infusion period. All subclass means are ranked in the same order for all patches for the 28 Sept. sampling showing that there was no interaction at that time.

There is no indication of significant interaction between the cysteine treatment effect and the stage of the seasonal

Table (4.10). Showing the Subclass Means of Each Body Region and Their Ranking.

Sampling	Ranking							
	Right mid-side		Left mid-side		Shoulder		Rump	
7 Sept.	HC	649.5	HS	658	HC	969	HC	933
	LC	597	HC	644	HS	717	HS	702.3
	HS	556	LC	510	LC	626.6	LC	674
	LS	378.3	LS	452	LS	431.3	LS	420.6
28 Sept.	HC	675	HS	650	HC	945	HC	886
	HS	601.6	HC	670.3	HS	768	HS	754.6
	LC	551.3	LC	505	LC	651.6	LC	634
	LS	436.3	LS	435.6	LS	458.3	LS	386.6

wool growth cycle. But insufficient data was obtained from this experiment to exhaustively test this possibility. When the adjusted means of the 15 June 2.0 gm. treatment class and the 7 Sept. HC subclass are compared with their respective saline control adjusted means, increases of 46.3% and 40.49% are found. A larger discrepancy was noted at the post-experimental samplings but this difference is probably not significant.

(b) fibre diameter response: the mean fibre diameter of the samples taken from the right mid-side patch at the end of the infusion and post-experimental periods are shown plotted against the corresponding mean daily wool growth figures in Fig. (4.5). Analyses of covariance of mean fibre diameter (x) and wool growth rate (y) are shown in Tables (4.11) and (4.12).

The Between Subclass regressions, and the Within Subclass regression for 28 Sept. are not equal to zero. The standard errors of these regressions are less than the regression coefficients, thus showing that in these cases wool growth rate and fibre diameter are positively correlated. The Within Subclass regression for 7 Sept. is not different from zero (b_{xy} = 9.0979, S.E. = $\pm$ 11.16). This was due to a greater scattering of principally the LS and HS data (see Fig. 4.5).

The high positive regression coefficients show that, again, an increase in fibre diameter contributed to the observed wool growth rate increases.

The standard deviations of fibre diameter were treated as

Table (4.11). Regressions of Mean Fibre Diameter (x) against Wool Growth Rate (y) for Data Taken at the End of the Infusion Period (7 Sept.).

Source	df	x^2	xy	y^2	b_{xy}	df	$(b_{xy})(xy)$	Σ d
Total	10	227.2364	4736.1546	189790.182	20.8424	9		
B. Subclass.	3	134.3964	3891.5046	112703.015	28.9554	2	112680.0723	22.9427
W. Subclass.	7	92.8400	844.6500	77087.167	9.0979	6	7684.5412	69402.6258

S.E.'s of Regressions:

B. Subclass = $\sqrt{0.08535}$ = ±0.29

W. Subclass = $\sqrt{124.5918}$ = ±11.16

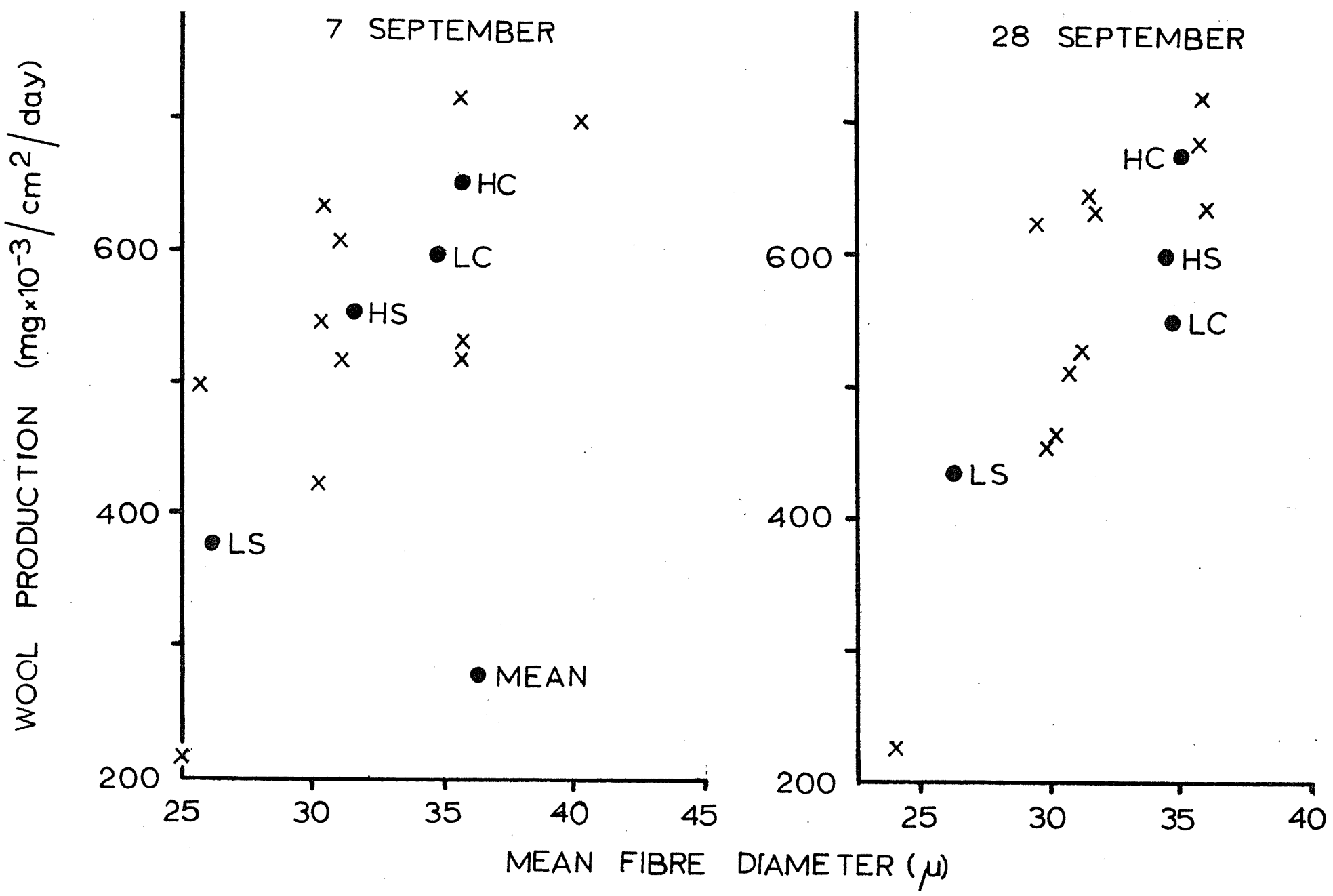
Table (4.12). Regressions of Mean Fibre Diameter (x) against Wool Growth Rate (y) for Data Taken at the End of the Post-experimental Period (28 Sept.).

Source	df	x^2	xy	y^2	b_{xy}	df	$(b_{xy})(xy)$	Σd
Total.	10	179.3564	4707.2637	198351.637	26.2453			
B. Subclass.	3	103.9264	2587.1137	77603.6369	24.8937	2	64402.8323	13200.8046
W. Subclass.	7	75.4300	2120.1500	120748.0001	28.1075	6	59592.1161	61155.8840

S.E.'s of Regressions:

B. Subclass. = $\sqrt{63.5105}$ = ±7.97

W. Subclass. = $\sqrt{135.1272}$ = ±11.62


7 SEPTEMBER
28 SEPTEMBER
WOOL PRODUCTION (mg×10⁻³/cm²/day)
MEAN FIBRE DIAMETER (μ)
HC
LC
HS
LS
MEAN
600
400
200
25
30
35
40
45

in the dose level response experiment and are shown in Fig. (4.6). Both the fibre diameters and the standard deviations follow the same trends, indicating that the diameters of all fibres have increased to the same extent.

(c) the effect of cysteine infusion on the digestibility of the basal feed: the crude protein and dry matter digestibilities determined during the period of cysteine infusion are listed in Table (4.13).

Analyses of variance (see Tables 4.14 and 4.15) indicate that cysteine infusion may have increased the crude protein digestibility but probably did not affect the dry matter digestibility.

The two diets differed markedly in dry matter and crude protein digestibility. It is of interest that when these digestibility coefficients are compared with those originally determined for the feeds, the HC subclass means for both dry matter and crude protein, and the low protein feed dry matter figures are similar; but the others are all lower than the original digestibilities. This may be due to error, or perhaps to a deleterious effect of saline infusion on crude protein (and possibly dry matter) digestibility.

(d) the effect of cysteine infusion on liveweight: the animals liveweight responses are shown by subclass means in Fig. (4.7). A possible treatment response is indicated but this is very small (the animals individual weights deviate by no more than 2.5 Kg. from their whole-experiment mean) and could be due to differences in gut fill at the various weighings.

Table (4.13). Mean Crude Protein and Dry Matter Digestibilities (expressed as %).

	High Protein Feed		Low Protein Feed	
	CP	DM	CP	DM
Cysteine	64.17	63.62	52.33	49.92
	60.12	58.78	51.84	50.71
			50.07	52.04
Mean	62.15	61.20	51.41	50.56
Saline	61.59	61.54	49.06	50.35
	55.75	55.55	59.93	52.46
	56.84	55.65	41.58	46.18
Mean	58.06	57.58	49.99	49.66

Table (4.14). Analysis of Variance of Crude Protein Digestibility Data.

Source	SS	df	ms	F
Total	420.5967	10	42.0597	
B. Diets.	314.9863	1	314.9863	27.82 ***
B. Treats.	46.5904	1	46.5904	4.11 * (p=0.07)
Interact.	0.0	1		
W. Subclass.	79.2702	7	11.3243	

Table (4.15). Analysis of Variance of Dry Matter Digestibility Data.

Source	SS	df	ms	F
Total	284.8153	10	28.4815	
B. Diets.	224.3246	1	224.3246	27.09 ***
B. Treats.	15.8776	1	15.8776	1.92 N.S.
W. Subclass.	57.9625	7	8.2808	
Interact.	0	1		

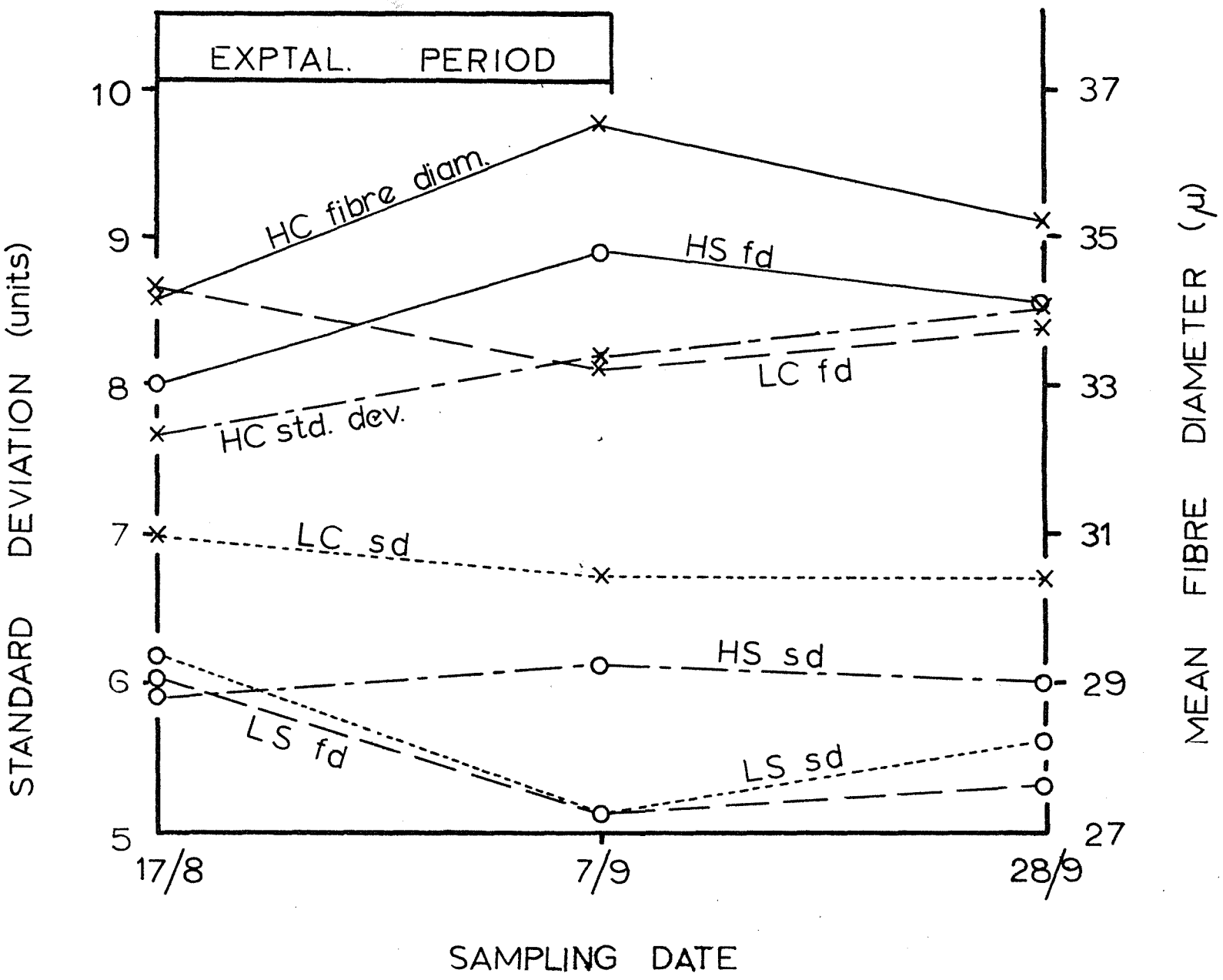
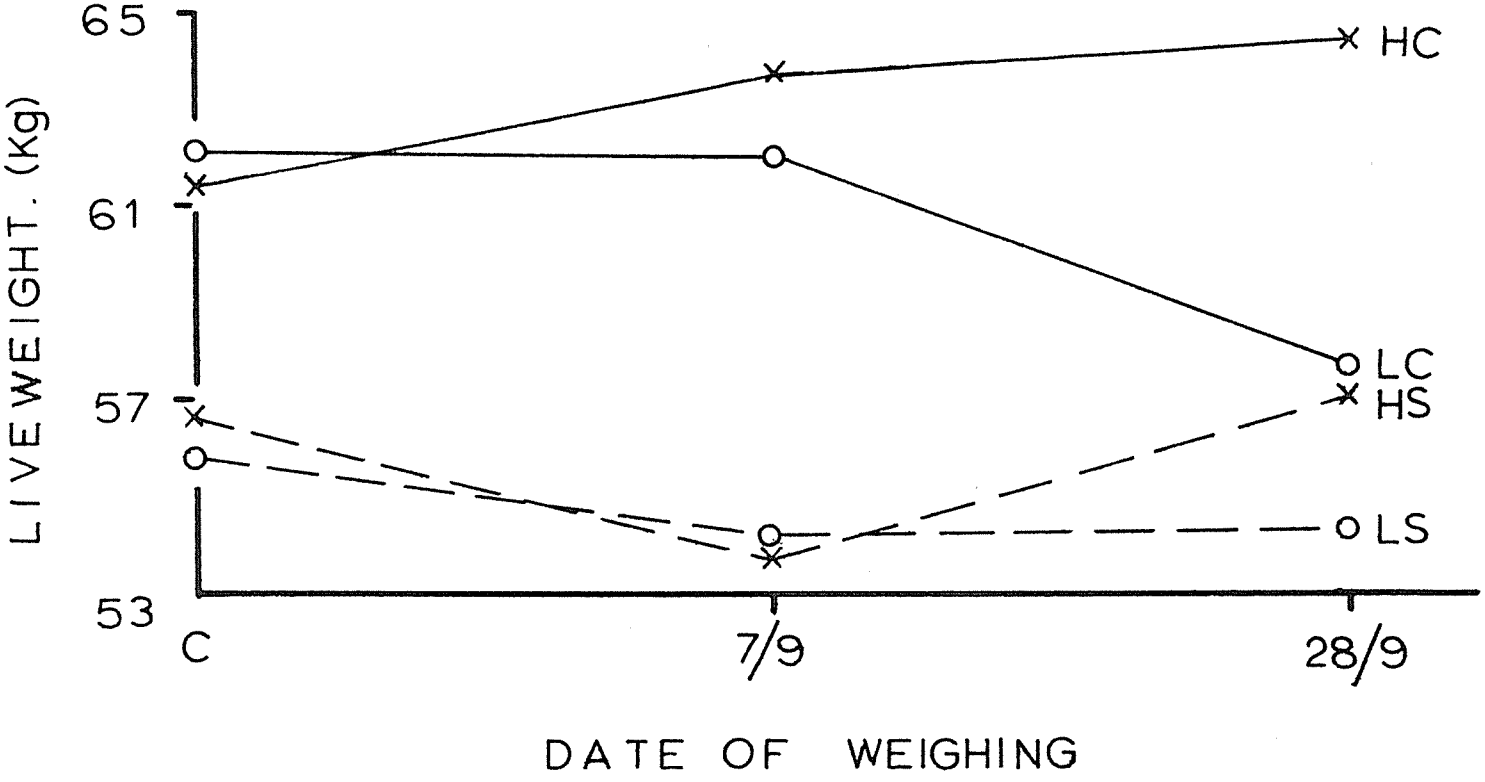

EXPTAL. PERIOD
HC fibre diam.
HS fd
LC fd
HC std. dev.
LC sd
HS sd
LS fd
LS sd
STANDARD DEVIATION (units)
MEAN FIBRE DIAMETER (μ)
10
9
8
7
6
5
37
35
33
31
29
27
17/8
7/9
28/9
SAMPLING DATE

FIG. (4.7). LIVEWEIGHT RESPONSES TO CYSTEINE INFUSION.



CHAPTER 5. DISCUSSION.

General:

While the results of this work and of the Australian work are similar, it is possible that the difference in infusion technique could preclude a direct comparison of the experiments.

Flow charts for the two infusion techniques are shown in Figs. (5.1) and (5.2).

Fig. (5.1). Proposed Flow Chart for Abomasally Infused Cysteine.

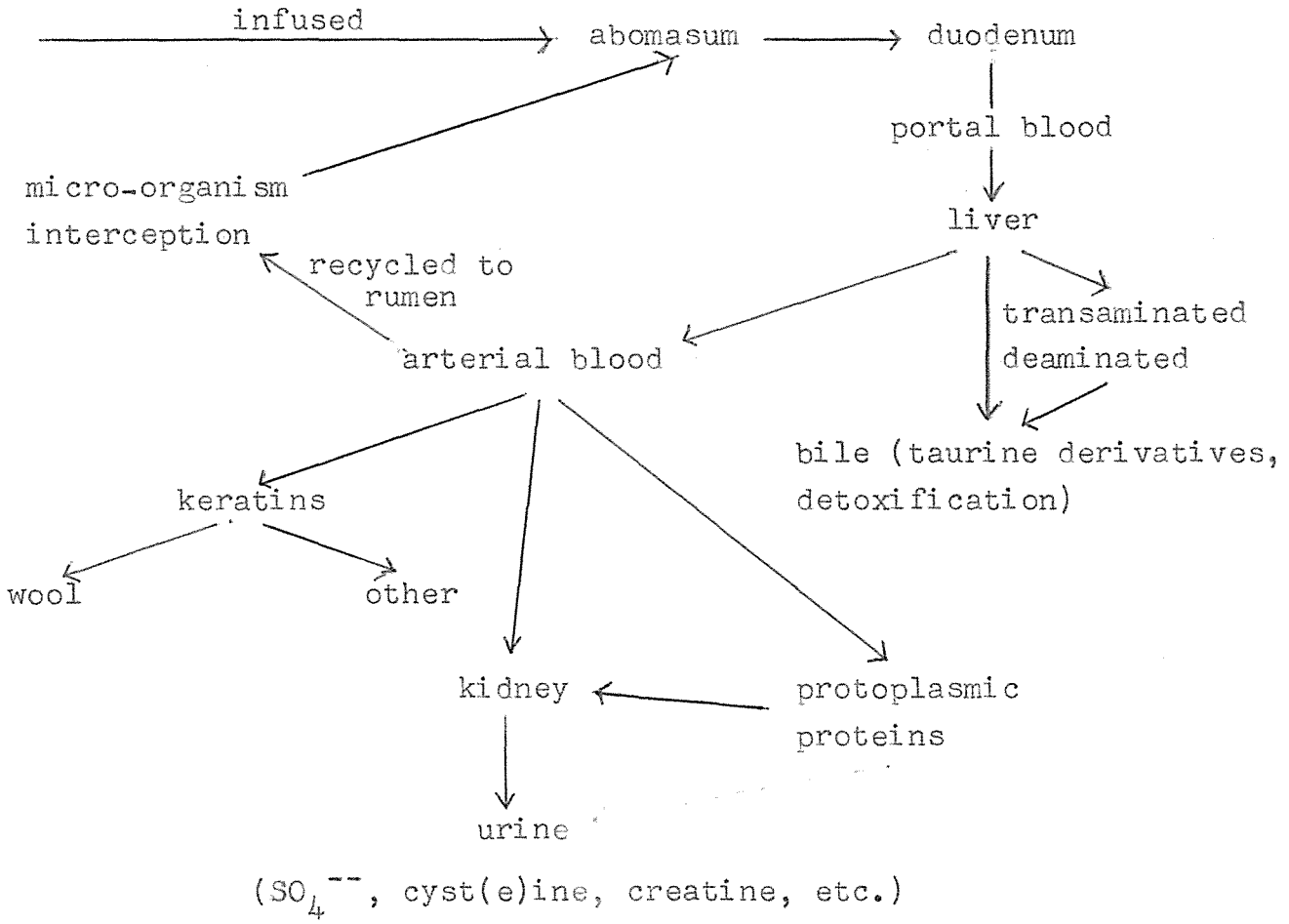
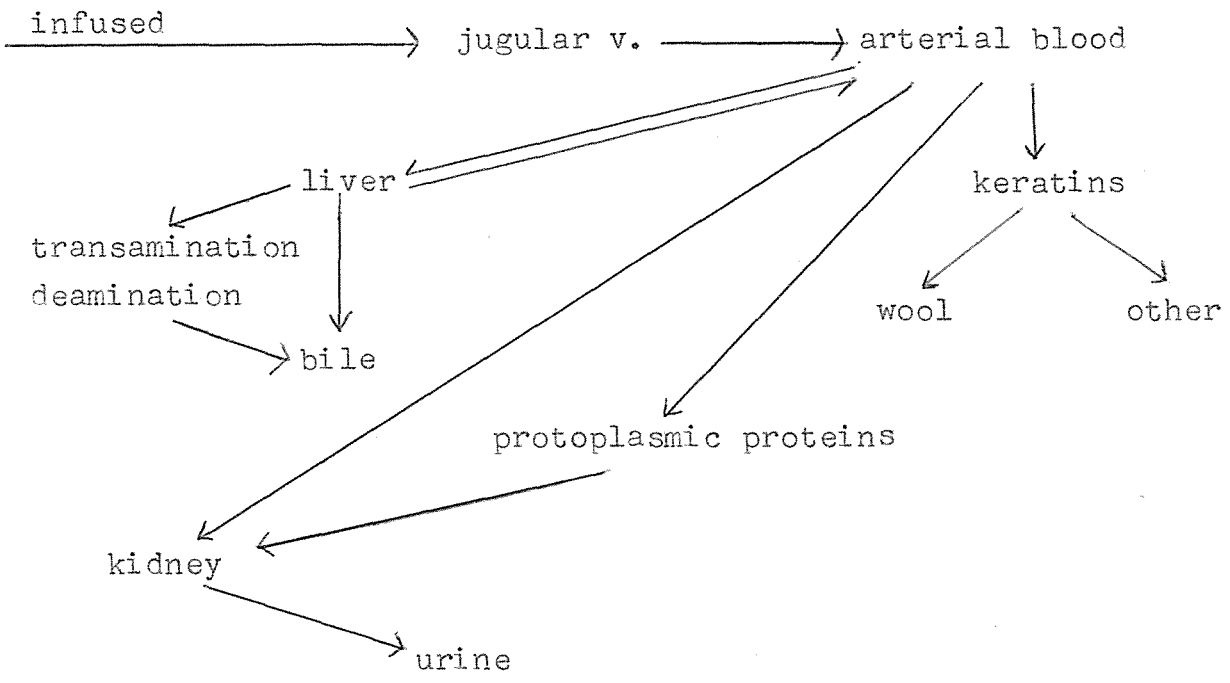


Fig. (5.2). Proposed Flow Chart for Venous Infused Cysteine.



The main difference is that abomasally infused sulphur containing amino-acid is presented to the liver before it reaches the peripheral tissues and so is exposed to possible degradation before it reaches the follicle. Most venous infused cysteine, however, reaches the tissues before the liver and so, in a situation of adequate sulphur supply, a greater proportion of infused cysteine could possibly be available for wool production. In a state of poor sulphur nutrition there will probably be no deamination in the liver and so no difference in the volume of sulphur containing amino-acid being presented to the follicle. It is probable that there are no differences between the Australian and this work as far as the utilisation of cysteine for wool growth is concerned as the diets fed by Reis and Schinkel did not supply sulphur containing amino-acid in excess of the requirements calculated by Downes (1961b).

An unexpected side-effect of the experimental technique was a loss of appetite in those animals receiving saline alone. The inappetance was marked and could have contributed to the decline in wool growth rate shown by animals numbers 6 and 10 in the dose level response experiment (see Fig. 4.1). This effect was noted in only animal number 4 (LS) in the protein level experiment. It is not known why the infusion of saline should lead to inappetance as the animals did not appear to be affected by water intoxication, although the effect is sometimes seen in human patients receiving this treatment (Laney, pers. comm.). It has also been suggested that cysteine may stimulate appetite; so the results obtained in the dose level response experiment could perhaps be in part, a result of differences in protein intake.

Experimental Results:

Wool growth rate increases of 40% to 130% were obtained in the present experiments and were characterised by a delay of about two weeks in their appearance, and a continuation of three to four weeks after the end of infusion. The duration of these were consistent over the present work and with the Australian observations.

Ryder (1958) and others have found that cyst(e)ine is incorporated into the wool fibre at the prekeratogenous region within 30 minutes of injection. This would appear to conflict with the results of the infusion experiments but it must be remembered that these workers were investigating the incorporation of cyst(e)ine into the fibre, rather than any possible increase in wool growth rate.

Downes (1961a; 1961b) and Downes, et al. (1964) found evidence for the binding of cysteine to the plasma proteins and suggested that this may constitute a 'pool' of this amino-acid. It is possible that either the replenishment of this pool is the first use for infused cysteine, or that wool growth may be buffered by it against fluctuations in the supply of sulphur containing amino-acids (Reis and Schinkel, 1963). The continuation of response suggests that the response delay may be caused by this pooling of cysteine and its release over a later period of time.

Reis and Schinkel (1963) suggest that the delay in response may be a function of the time necessary for the wool to emerge from the skin and appear in the clippings. Wickham (pers. comm.) suggests that only three days elaspse between the production of a germinal tissue cell and its appearance at the skin surface. Further, an increase in the rate of fibre production in the bulb should immediately manifest itself as an increase in the rate of fibre emergence at the skin surface.

The administration of 2.0 gm. per day of extra cysteine represents an increase of about 100% in the animals cysteine intake. It is possible that the follicle may not be immediately adapted to utilise the extra subtrate.

Further, the demand for sulphur containing amino-acids is not confined to that for wool production. The thiol-dithiol exchange reaction is used in a great may biochemical processes, and the sulphur containing amino-acids (or parts of them) are found in many substances synthesised in the body. Where a sheep

is in a negative or zero sulphur balance it is quite possible that these other manifold requirements for cyst(e)ine take precedence over that for wool growth. Mitchell (1959) states that the proptoplasmic tissues may be raided for the amino-acids necessary for keratin sythesis. This suggests that supplementary cysteine may first be used for the nutrition of the protoplasmic tissues rather than being made available to the wool follicle.

The dose level response results of this work agree with those of the Australian experiments described in Chapter 2. Reis (1967) showed that while small amounts of sulphur containing amino-acids increase the wool growth rate by up to 100%, larger doses (3.0 to 8.0 gm. per day) gave smaller responses. From the experiment carried out in this series, it would seem that the optimum cysteine dose for the New Zealand Romney is similar to that of the Australian breeds tested, as a slightly (but not significantly) smaller response was obtained from 4.0 gm. cysteine per day than from 2.0 gm. per day.

It has been calculated (Downes, 1961b) that sheep require about 3.1 gm. sulphur containing amino-acid per day, and suggested that any excess would be excreted. While Downes's figures are, for the most part, approximate, it is not unlikely that part of a high dose of cysteine would be excreted. However, a comparison of the figures calculated for sulphur containing amino-acid content of the high protein diet fed in the present work and of the Australian diets suggests that the efficiency of utilisation of administered cyst(e)ine depends on the amino-acid balance of the diet and the animals ability to utilise excess cyst(e)ine, as well as the basal requirement for the animals normal metabolic processes.

Evidence has been obtained in this work and suggested in that of Reis (1967) that the sulphur containing amino-acid content of the basal diet affects the efficiency of utilisation of infused cysteine. This is most evident from the second experiment of this series where a pronounced interaction between dietary protein content and cysteine treatment occured.

In this experiment the high protein feed contained about 16% crude protein and so supplied about 5.3 gm. sulphur containing amino-acid per day, a figure higher than that of the Australian diets (2.0 to 3.0 gm. per day). The low protein diet had a crude protein content of about 11%, supplying about 3.6 gm. sulphur containing amino-acid per day. Although no data is obtainable about the digestibility of these amino-acids it is probable that the diet x cysteine treatment interaction was due to the differing sulphur containing amino-acid content of the basal diets.

The interaction shows that the effect of cysteine on wool production is related to the protein nutrition of the animal: cysteine either supplementing the sulphur containing amino-acid content of the basal diet or stimulating the digestion of dietary protein (and possibly carbohydrate). It is more probable that the infused cysteine supplemented the basal content, as a stimulation of digestion, would be expected to give greater wool growth rate increases from that diet capable of benefiting most from such an effect, viz. the high protein diet. However, the increase in protein digestibility due to cysteine infusion suggests that this mechanism may also have been involved. It is not likely, though, that the small increase in digestibity obtained would, of

itself, lead to wool growth rate increases of the magnitude observed. It should be noted that the different diets gave less significant wool growth rate differences than did the cysteine treatment.

The 'supplementation' of dietary protein referred to above could occur when extra sulphur containing amino-acids are given to the sheep. The amino-acid balance of a protein limits the efficiency of utilisation of that protein; and if one amino-acid is limiting for a particular metabolic function (as cysteine could be for wool production) then its administration will improve the amino-acid balance of digested protein and will allow the utilisation of a greater proportion of the absorbed amino-acids.

The possibility of nitrogen and sulphur recycling to the rumen via saliva can not be discounted and would lead to an increase in the activity of the rumen micro-organisms (and hence to improved feed digestibility), and to an increase in the biological value of microbial protein.

No increase in dry matter digestibility was obtained in this experiment although Reis and Schinkel (1963; 1964) and Moir and Harris (1962) have obtained such increases from abomasal casein infusion. On theoretical grounds one would be expected: if feed digestibility in the rumen is enhanced by nitrogen or sulphur recycling then this would be expected to affect dry matter digestion more than protein digestion.

It should be noted that the sheep fed the low protein diet

received about 160 gm. per day less digestible dry matter than those fed the high protein diet. Marston (1948) has calculated that only about 4% of the sheeps digestible energy intake is used for wool production, and Graham (cited by Reis and Schinkel, 1963) obtained a wool growth rate increase of only 17% from an increase in metabolisable energy of 450 Kcal. per day. The sheep fed the high protein diet could have received at the most only 640 Kcal. metabolisable energy per day more than those fed the low protein diet (using a factor of 4.0 Kcal. / gm. DDM given by Blaxter, 1962), so it is unlikely that the difference in digestible dry matter intake between the two diets would lead to either the between diet wool growth rate difference found, or to the diet x cysteine treatment interaction.

The results obtained in the present experiments confirm the results of Reis and Schinkel (1960) and Schinkel (1962) who found that increased fibre diameter was a component of the wool growth rate response.

No evidence for a differential effect of sulphur containing amino-acid administration on either different fibre types or on fibres of different diameter was presented in the Australian papers. The Romney has a lower S/P ratio than the Merino and has wide variation in the diameter of different fibres. It would be expected that a pelage of this type would show up a differential effect, but little evidence for such an effect was obtained.

Both fibre diameter and the sample standard deviations of ~~the~~

fibre diameter followed similar trends at both stages of the seasonal wool growth rate cycle tested (see Figs 4.3 and 4.6). It is, therefore, unlikely that increased cysteine availability interacted with the seasonal effects, especially as there was no apparent wool growth rate interaction of this type.

The sample patch subclass means obtained at the end of the infusion period indicate a possible body-region x cysteine treatment interaction. It is noted, though, that the mid-side patch subclass means obtained at the 28 September sampling became ranked in the 7 September order when they were adjusted to the mean of the pre-experimental control wool growth rate values; so it is possible that the out-of-order shoulder and rump rankings would become similarly re-ordered if adjusted to their respective pre-experimental control values. If an interaction does exist, it may possibly be explained after reference to the S/P ratio and dP/dS ratio gradients over the body.

A comparison of the present experiments and those of Reis (1967) suggests that the New Zealand Romney and the Australian Merino respond similarly to sulphur containing amino-acid administration. For example, compare the 130% increase in wool growth rate obtained from the LC subclass, with the approximately 100% increase obtained by Reis, (1967) at the same dose level.

The Mechanism of the Cysteine Effect:

Short, et al. (1965) showed that the wool growth rate response

to casein infusion occurred in two way: by an increase in the number of proliferating cells in the germinal tissue, and by an increase in the rate of proliferation of these cells. A slight increase (about 4%) was obtained in the proportion of cells entering the cortex, and some (about 20%) increase in the volume of cortical and germinal tissue cells (which may have been related to an increase in the high-sulphur protein content of these cells). It is concluded then, that the major factors responsible for the increase in wool production were the two first mentioned.

Excess cysteine may increase the germinal tissue cell production rates in two ways:

(i) by increasing the amount or activity of chemotransmitters affecting mitosis, or

(ii) by increasing the amount of cysteine, per se, or of other amino-acids, for use as substrate for cell production.

The mechanisms which may bring about an increase in amino-acid substrate for cell production have been described in Chapter 2 and earlier in this Chapter. To these mechanisms may be added the suggested stimulatory effect of cyst(e)ine on feed intake, although this mechanism has not yet been proven to exist.

Reis and Schinkel (1963) suggested a 'general anabolic effect' and cited as evidence an increase in liveweight in those

animals receiving cysteine. No possible mode of action was postulated but an increase in chemotransmitter activity is a possible mechanism, but probably not the sole one. It is suggested that the effects of cysteine administration on amino-acid utilisation may have been responsible for the increase in liveweight noted. It was assumed by Reis and Schinkel (1963) that the mechanism causing increased liveweight is the same as that causing increased wool growth rate, but this need not be so as Reis (1967) found liveweight increases continuing at dose levels sufficient to reduce wool production to below the control level.

No strong evidence of liveweight increases was found in the present experiments. It is not likely that the activation of chemotransmitters differs between Romneys and Merinos, so the lack of a liveweight increase in the present experiment may have been due to differences in the effect of cysteine on the utilisation of amino-acids between the diets used.

The production of a wool fibre also involves the keratinisation of the cells produced in the germinal tissue. This involves the formation of filamentous protein (which has a low sulphur content), and the subsequent formation of the higher-sulphur matrix protein. It is not know how the matrix is formed, but it is probably by either the synthesis of a new protein or by the incorporation of cyst(e)ine into the low-sulphur helices.

It was suggested (Reis and Schinkel, 1964) that the synthesis

of high-sulphur protein may be the rate limiting step in keratin synthesis; and it has been shown (Gillespie, et al. 1964; Gillespie, 1965) that changes in the sulphur content of wool following cyst(e)ine administration are due to changes in the proportion of high-sulphur protein. However, it has also been shown that wide fluctuations in sulphur content are not necessarily accompanied by similar fluctuations in wool growth rate (see Fraser, 1967; Reis 1967). Consequently, the synthesis of high-sulphur protein is probably not the factor regulating wool growth.

It is not known whether the protein constituent of the newly formed germinal tissue cell is the same as that of the filamentous protein, or whether a new low-sulphur protein is synthesised as the cell moves up the follicle. In either case the formation of filamentous protein requires the supply of the necessary amino-acids, and the administration of cysteine may increase this supply, as was noted earlier.

Casein supplies amino-acids other than cyst(e)ine and this may explain its greater stimulatory effect on wool production, noted in Australian work and by Edwards (pers. comm.) with the New Zealand Romney.

Reis and Schinkel (1963) suggest that infused cysteine may stimulate the production of filamentous protein by supplying increased amounts of thiol groups. However, as workers (see Chapter 2) have shown that infused cysteine probably enters

the follicle at the prekeratogenous zone this theory assumes that these groups can be incorporated into the already formed filamentous protein. De Bevasques and Rothman (1962) suggest that this does not happen, Mercer (1961) states that the filaments are completely formed at the prekeratogenous stage, and evidence from infusion experiments (see above) suggests that most incorporated cysteine appears in the matrix protein. On the other hand, the evidence of Nakai (1964) and Downes, et al. (1966) suggests that injected cysteine can be incorporated into the filaments.

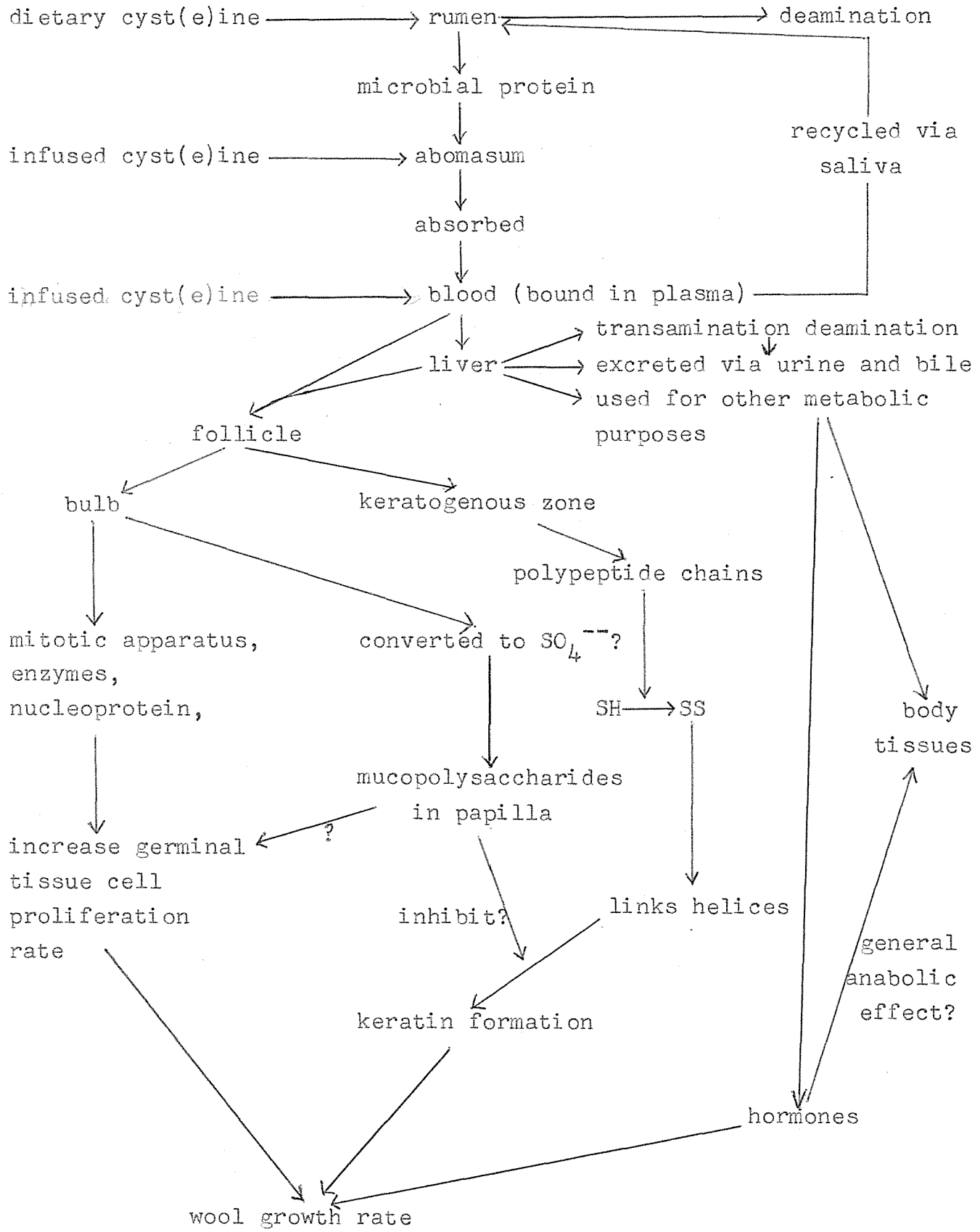
As far as wool growth is concerned, the metabolism of cyst(e)ine can be shown as in Fig. (5.3).

In summary, the probable uses for infused cyst(e)ine insofar as wool production is concerned, are:

(i) to increase the proportion of the high-sulphur proteins, an action which increases the sulphur content of the fibre but probably not its growth rate, and

(ii) to increase the rate of synthesis of filamentous protein and the rate of proliferation of cells in the germinal tissue. It is suggested that the 50% to 85% of infused cyst(e)ine not accounted for in the fibre may, in part, be used for the stimulation of mitosis in the germinal tissue and that this effect of the administration of sulphur containing amino-acids is the more important insofar as wool production is concerned.

Fig. (5.3). The Metabolism of Cyst(e)ine For Wool Growth.



SUMMARY.

Two experiments are reported:

(a) an investigation into the wool growth rate response to dose levels of 2.0 gm. and 4.0 gm. L-cysteine per day; and

(b) an investigation into the effects on wool growth rate, liveweight and the digestibility of the basal diet of the administration of 2.0 gm. L-cysteine per day superimposed on two basal dietary protein levels.

The wool growth rate responses were characterised by a consistent two-week delay in appearance. The responses continued for about three weeks after the end of the infusion period.

A differential dose level response was indicated: mean wool growth rate increases of 53.51% and 83.87% over the control wool growth rates were obtained at the ends of the infusion and post-experimental periods respectively, from those animals receiving 2.0 gm. per day; and increases of 40.74% and 80.53% respectively from those receiving 4.0 gm. per day.

A pronounced diet x cysteine treatment interaction was found: the mean wool growth rate of the high protein-cysteine subclass was increased by 40.49% over the high protein-saline mean at the end of the infusion period, while the low protein-cysteine subclass mean wool growth rate increased by 130.23%

over the low protein-saline subclass mean. Mean increases of 40.31% and 99.98% were obtained from the high protein-cysteine and low protein-cysteine subclasses respectively, at the end of post-experimental period.

A slight (non-significant) increase in crude protein digestibility was obtained, but no increase was found in dry matter digestibility.

There was little indication of a liveweight response to cysteine administration.

Increases in mean fibre diameter contributed to all the observed increases in wool growth rate, and there was probably no differential effect of cysteine treatment on different fibre types.

The existence of a possible body-region x cysteine treatment interaction attributable to S/P and dP/dS ratio gradients is discussed.

Only slight evidence for a season x cysteine treatment interaction was found for either the wool growth rate or fibre diameter responses.

The results obtained are discussed, and possible mechanisms of the responses to cysteine administration are examined.

BIBLIOGRAPHY.

1. ANNISON, E.F. and LEWIS, D. (1959): 'Metabolism in the Rumen' (Methuen, London).

2. BERN, H.A. (1954): Nature, 174, 509.

3. BERN, H.A., HARKNESS, D.R. and BLAIR, S.M. (1955): Proc. Nat. Acad. Sci. U.S., 41, 55.

4. BLAXTER, K.L. (1962): 'The Energy Metabolism of Ruminants' (Hutchinson, London).

5. BLOCK, R.J., STEKOL, J.A. and LOOSLI, J.K. (1951): Arch. Biochem. Biophys., 33, 353.

6. BLOCK, W.D., MARKOVS, Mara E., and STEELE, Betty F. (1965): J. Nutr., 86, 256.

7. BOWSTEAD, J.E. and LAROSE, P. (1938): Canad. J. Res., 16D, 361.

8. BRAUN-FALCO, O. (1958): in 'Biology of Hair Growth', ed. W. Montagna and R.A. Ellis (Academic Press, New York).

9. BURNS, M. and CLARKSON, H. (1949): J. Agr. Sci., 39, 315.

10. BURROUGHS, W., ARIAS, C., DePAUL, P. GERLAUGH, P. and BETHKE, R.M. (1951): J. Animal Sci., 10, 672.

11. CAVALLINI, D., MONDOVI, B. and De MARCO, C. (1955): J. Biol. Chem., 216, 577.

12. CHALMERS, Margaret I. (1961): in 'Digestive Physiology and Nutrition of the Ruminant', ed. D. Lewis (Butterworths, London).

13. CHAPEVILLE, F., FROMAGEOT, P., BRIGELHUBER, A and HENRY, M. (1956): Biochim. Biophys. Acta, 20, 351.

14. COETZEE, C.G. and PIETERSE, P.J.S. (1966): S. African J. Agr. Sci., 9, 889.

15. COHEN, P.P. (1940): J. Biol. Chem., 136, 565.

16. CREWTHER, W. G. and HARRAP, B.S. (1965): Nature 207, 295.

17. CUTHBERTSON, Sir D. (1965): ed. 'The Nutrient Requirements of Farm Livestock, No. 2 Ruminants, Technical Reviews and Summaries (Agricultural Research Council, London).

18. DALY, R.A. and CARTER, H.B. (1955): Australian J. Agr. Res., 6, 476.

19. De BERSAQUES, J. and ROTHMAN, S. (1962): Nature, 193, 147.

20. DOWNES, A.M. (1961a): Australian J. Biol. Sci., 14, 109.

21. ———————— (1961b): Ibid, 14, 427.

22. ————————, FERGUSON, K.A., GILLESPIE, J.M. and HARRAP, B.S. (1966): Ibid, 19, 319.

23. ————————, LYNE, A.G. and CLARKE, W.H. (1962): Ibid, 15, 713.

24. ————————, SHARRY, L.F. and TILL, A.R. (1964): Ibid, 17, 945.

25. ———————————————————————— (1965): Ibid, 18, 140.

26. DUNCAN, D.B. (1955): Biometrics, 11, 1.

27. Du TOIT, P.J., MALAN, A.I., GROENEWALD, J.W. and BOTHA, P.S. (1935): Onderstepoort J. Vet. Sci. Animal Ind., 4, 229.

28. Du VIGNEAUD. V., DORFMANN, R. and LORING, H.S. (1932): J. Biol. Chem., 98,

29. Du VIGNEAUD, V., MOYER, A.W. and CHANDLER, J.B. (1944): Ibid, 174, 477.

30. EAGLE, H., OYAMA, V.I. and PIEZ, K.A. (1960): Ibid, 235, 1719.

31. EDWARDS, L.J. (1954): Biochem. J., 57, 542.

32. ELLIS, W.C., GARNER, G.B., MUHRER, M.E. and PFANDER, W.H. (1956): J. Nutr., 60, 413.

33. EVVARD,

34. FERGUSON, K.A. (1959): Nature, 184, 907.

35. ————————, CARTER, H.B. and HARDY, M.H. (1949): Australian J. Sci. Res., B2, 42.

36. FRASER, I.E.B. (1967): 'A Review on the Utilisation of Fodder and Supplementary Feeding of Crude Proteins and Sulphur-Containing Amino-Acids to Sheep for Increased Wool Production' (Wool Research Organisation of New Zealand (Inc.), Communication No. 1.).

37. FRASER, A.H.H. and ROBERTS, J.A.F. (1933): J. Agr. Sci., 23, 97.

38. FROMAGEOT, C. (1951): 'The Enzymes', 2, ed. J.B. SUmner and K. Myrback (Academic Press, New York).

39. GILLESPIE, J.M. (1965): in 'Biology of the Skin and Hair Growth' ed. A.G. Lyne and B.F. Short (Angus and Robertson, Sydney).

40. ————————, REIS, P.J. and SCHINKEL, P.G. (1964): Australian J. Biol Sci., 17, 548.

41. HALE, W.H. and GARRIGUS, U.S. (1953): J. Animal Sci., 12, 492.

42. HAMILTON, T.S., ROBINSON, W.B. and JOHNSON, B.C. (1948): Ibid, 7, 35.

43. HARKNESS, D.R. and BERN, H.A. (1957): Acta Anat., 31, 35.

44. HARPER, A.E. (1958): Ann, N.Y Acad. Sci., 69, 1025.

45. HARRIS, L.E. and MITCHELL, H.H. (1941): J. Nutr., 22, 167.

46. HARTWELL,

47. HARVEY,

48. HAWLEY, E.E., MURLIN, J.R, NASSET, E.S. and SZYMANSKI, T.A. (1948): J. Nutr., 36, 153.

49. HOLMES, P., MOIR, R.J. and UNDERWOOD, E.J. (1953): Australian J. Biol Sci., 6, 637.

50. HOPE, D.B. (1955): Biochem. J., 59, 497.

51. JENSEN, E.V. (1959): Science, 130, 1319.

52. JOHNSON, B.C., HAMILTON, T.S., MITCHELL, H.H. and ROBINSON, W.B. (1942): J. Animal Sci., 1, 236.

53. ———————————————, ROBINSON, W.B. and GAREY, J.C. (1944): Ibid, 3, 287.

54. KALAN, E.B. and CEITHAML, J. (1954): J. Bacteriol., 68, 293.

55. KEHAR. N.D. and MUKHERGEE, R. (1949): Indian J. Vet. Sci., 19, 283.

56. KEILIN, D. (1930): Proc. Roy. Soc. (London), Ser. B, 106, 418.

57. KING, A.T. and NICHOLS, J.E. (1933): Trans. Faraday Soc., 29, 272.

58. KREBS, H.A. (1964): in 'Mammalian Protein Metabolism', ed. H.N. Munro and J.B. Allison (Academic Press, New York and London).

59. KUN, E. (1961): in 'Metabolic Pathways', II, ed. D.M. Greenberg (Academic Press, New York and London).

60. KWONG, E. and BARNES, R.H. (1963):

61. LEE, N.D., ANDERSON, J.T., MILLER, R. and WILLIAMS, R.H. (1951): J. Biol. Chem., 192, 733.

62. LEROY, A.M. and CHARTELET, P.V. (1949): Nutr. Abstr. Rev., 19, 485.

63. LEWIS, D. (1954): Biochem. J., 56, 391.

64. ———— and ELSDEN, S.R. (1955): Ibid, 60, 683.

65. LITTLE, C.O. and MITCHELL, G.E., Jnr. (1967): J. Animal Sci., 26, 411.

66. LOFGREEN, G.P., LOOSLI, J.K. and MAYNARD, L.A. (1947): Ibid, 6, 343.

67. McDONALD, I.W. (1954): Biochem. J., 56, 120.

68. ———————— and HALL, R.J. (1957): Ibid, 67, 400.

69. McNAUGHT, M.L., SMITH, J.A.B., HENRY, K.M. and KON, S.K. (1950): Ibid, 46, 32.

70. MARSTON, H.R. (1932): Australia, Council for Scientific and Industrial Research, Bulletin No. 61.

71. ———————— (1935): J. Agr. Sci., 25, 113.

72. ———————— (1948): Australian J. Sci. Res. B1, 93.

73. MAZIA, D. (1959): in 'Sulfur in Proteins', ed. R. Benesch, et al. (Academic Press, New York).

74. ———————— (1961): in 'The Cell', 3, ed. J. Brachet and A.E. Mirsky (Acadamic Press, New York).

75. MEDES, G. (1939): Biochem. J. 33, 1559.

76. ———————— and FLOYD, N.F. (1942): Ibid, 36, 259.

77. MEISTER, A. (1965): 'Biochemistry of the Amino Acids' (Academic Press, New York and London).

78. MERCER, E.H. (1961): 'Keratin and Keratinisation' (Pergammon Press, Oxford, London, New York and Paris).

79. ————————, MUNGER, B.L., ROGERS, G.E. and RØTH, S.I. (1964), Nature, 201, 367.

80. MITCHELL, H.H. (1959): in 'Protein and Amino Acid Nutrition', ed. A.A. Albanese (Academic Press, New York and London).

81. MOIR, R.J. and HARRIS, L.E. (1962): J. Nutr., 77, 285.

82. NAKAI, T. (1964): J. Cell Biol., 21, 63.

83. ORR, and HOLM (1931): Economic Advisory Council of Great Britain, Committee on the Mineral Content of Natural Pastures, 6th Report.

84. PIERCE, A.W. (1933): J. Council Sci. Ind. Res. Australia, 6, 294.

85. PIRIE, N.W. (1934): Biochem. J., 28, 305.

86. RACHELE, J.R., REED, L.J., KIDWAI, A.R., FERGER, M.F. and Du VIGNEAUD, V. (1950): J. Biol. Chem., 185, 817.

87. REIS, P.J. (1965): Australian J. Biol. Sci., 18, 671.

88. ———— (1967): Ibid, 20, 809.

89. ———— and SCHINKEL, P.G. (1961): Australian J. Agr. Res., 12, 335.

90. ———— (1963): Australian J. Biol. Sci., 16, 218.

91. ———— (1964): Ibid, 17, 532.

92. RYDER, M.L. (1958): Proc. Roy. Soc. Edinburgh, 67B 65.

93. SAMARINA, O.P., KRITZMAN, M.G. and KONIKOVA, A.S. (1956): Biochimia, 21, 10.

94. SCHINKEL, P.G. (1962): Animal Prod., 4, 122.

95. SHORT, B.F., WILSON, Patricia A. and SCHINKEL, P.G. (1965): in 'Biology of the Skin and Hair Growth', ed. A.G. Lyne and B.F. Short (Angus Robertson, Sydney).

96. SIROTNAK, F.M., DOETSCH, R.N., BROWN, R.E. and SHAW, J.C. (1953): J. Dairy Sci., 36, 1117.

97. SLEN, S.B. and WHITTING, F. (1952): J. Animal Sci., 11, 781.

98. SMUTS, S.B., MITCHELL, H.H. and HAMILTON, T.S. (1932): J. Biol. Chem., 95, 283.

99. SMYTHE, C.W. (1942): Ibid, 142, 387.

100. SNEDDON, H.R. and CHAMBERLAIN, W.E. (1933): Australian Vet. J., 9, 154.

101. SNEDECOR, G.W. (1956): 'Statistical Methods' (Iowa State University Press, Ames, Iowa).

102. SPÖTTEL, W. (1933): Nutr. Abstr. Rev., 3, 617.

103. STARKS, P.B., HALE, W.H., GARRIGUS, U.S. and FORBES, R.M. (1953): J. Animal Sci., 12, 480.

104. ———————————————————————————— and JAMES, M.F. (1954): Ibid, 13, 249.

105. STERN, H. (1959): in 'Sulfur in Proteins', ed. R. Benesch, et al. (Academic Press, New York).

106. STETTEN, D., Jnr. (1942): J. Biol. Chem., 144, 501.

107. STEYN, D.G. (1931): 17th Report of the Director of Veterinary Services and Animal Industry, S. Africa.

108. ———— (1932): 18th Ibid.

109. SYLVEN, B. (1950): Exp. Cell Res., 1, 582.

110. SYM, E.A. (1938): Acta Biol. Exp. Varsovie, 12, 192.

111. TARR, H.L.A. (1933): Biochem. J., 27, 1869.

112. THOMAS, W.E., LOOSLI, J.K., WILLIAMS, H.H. and MAYNARD, L.A. (1951): J. Nutr., 43, 515.

113. TSUKAMURA, S. (1964): Japan. J. Tuberc., 10, 15.

114. Van HORN, J.L., BURKETT, W.H., PAYNE, G.F., HUGHES, G.C. and WILSON, F.S. (1950): Montana Agr. Exp. Sta. Progr. Rep.

115. ———————————————————————————————————(1951): Montana Agr. Exp. Sta. Circular 56.

116. VIRTANEN, A.I. (1966): Science, 153, 1603.

117. WELLER, R.A. (1957): Australian J. Biol. Sci., 10, 384.

118. WILLIAMS, V.J. and MOIR, R.J. (1951): Australian J. Sci. Res., B4, 377.

119. WOODS. D.D. and CLIFTON, C.E. (1937): Biochem. J, 31, 1774.