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Changes in the Matrix Proteins of Wool and Mouse Hair following the Administration of Depilatory Compounds

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Abstract

After sheep were defleeced with mimosine, cyclophosphamide or *N*-[5-(4-aminophenoxy)pentyl]-phthalimide, the first samples of the new growth of wool differed markedly in composition from the pretreatment samples, there being substantial reductions in the high-tyrosine proteins and increases in the high-sulfur proteins. Similar results were obtained with mice dehaired with mimosine and with sheep treated with low levels of mimosine which resulted in weakened wool rather than depilation. The composition of later samples of the regrowth wool showed progressive changes with time. The high-tyrosine proteins tended to approach the pretreatment levels, although this may take up to 12 weeks to occur, whereas the levels of high-sulfur proteins, after the initial increase, often fell below normal. In experiments involving defleecing with cyclophosphamide, the level of the latter proteins was still below normal after 3 months.

The possibility that this altered protein composition of keratin fibres is characteristic of that portion of fibre first produced by a new or regenerating follicle was investigated in sheep and mice. It was found that wool follicles regenerating after plucking, and newly operating follicles in young sheep and mice, also produced wool and hair with a reduced content of high-tyrosine proteins. It is suggested, therefore, that the apparent long-term inhibition of the high-tyrosine proteins may not be the direct consequence of the administration of the chemical but rather be characteristic of normal wool and hair regrowth.

Infusion of an amino acid mixture lacking methionine into the abomasum of sheep caused the growth of weak wool but did not suppress the synthesis of the high-tyrosine proteins. This is in contrast with previous findings that treatments which weaken wool also suppress high-tyrosine proteins.

Introduction

In previous studies (Frenkel *et al.* 1974, 1975) it has been shown that the synthesis of the high-tyrosine proteins in wool follicles is substantially depressed by various treatments applied to sheep. These effects have been observed during the abomasal administration of methionine (to wheat-fed sheep), of amino acid mixtures lacking lysine or phenylalanine, of gluten proteins and of zein, and following the admisistration of the potential defleecing agents, mimosine and Opticortenol, given in amounts that did not completely stop wool growth. This partial suppression of the high-tyrosine proteins is usually accompanied by an increase in the relative proportions of the high-sulfur proteins (Frenkel *et al.* 1974, 1975). In the experiments with mimosine and Opticortenol there was an indication that the effects on the wool proteins persisted after wool growth rate had recovered (Frenkel *et al.* 1975). It is of interest that the rate of wool growth is often enhanced for several weeks after doses of mimosine have either reduced or temporarily stopped wool growth, but it is not known if these long-term effects are related (Reis 1975, 1978).

In the present study the effects of three chemical defleecing agents (mimosine, *N*-[5-(4-aminophenoxy)pentyl]phthalimide, and cyclophosphamide), and an amino acid infusion which weakens wool, on the matrix proteins (high-sulfur and high-tyrosine) of wool have been investigated. The defleecing agents were given in amounts sufficient to stop wool growth and allow the removal of the fleece; long-term effects on the high-tyrosine proteins in particular were studied during the regrowth of wool. Further experiments were carried out with sheep and mice in an attempt to provide an explanation of these long-term effects on the high-tyrosine proteins.

Mouse hair is considerably richer in high-tyrosine protein than most samples of wool (Gillespie and Frenkel 1974) and therefore might be expected to show greater effects following the administration of defleecing agents. Furthermore, due to its small size, the mouse might be a more convenient animal than the sheep for studies on the suppression of the high-tyrosine proteins following the administration of such compounds. Although it has been known for some time that mimosine has a depilatory effect on mice (Crounse *et al.* 1962; Montagna and Yun 1963) these animals are normally difficult to use in dehairing experiments because the cycles of hair growth are short and occur in waves. However, this problem is overcome if young mice still in the first hair growth cycle are used (Panaretto *et al.* 1978) and experiments have been carried out using this animal.

For the definition of certain terms used in this paper, e.g. wool weakening and defleecing, and for a review of chemical defleecing, the recent paper by Reis and Panaretto (1979) should be consulted.

Materials and Methods

Experimental Procedures with Animals

All sheep were kept indoors and dosed in ways as indicated below. Details of the diets and experimental treatments are given in the relevant references or in the description of each experiment. Sucking mice were maintained as described by Panaretto *et al.* (1978).

Experiment 1

Four Merino sheep were given intravenous infusions of L-mimosine (L-α-amino-β-(3-hydroxy-4-oxo-1,4-dihydro-1-pyridyl)propionic acid) daily for 2 days at a rate of 113–119 mg/kg body wt; see experiment 1 of Reis (1978).

Experiment 2

Five Merino sheep, consuming a daily ration of 800 g of a pelleted mixture of equal parts chopped lucerne and wheaten hays, were dosed with *N*-[5-(4-aminophenoxy)pentyl]phthalimide. One sheep received an intravenous infusion daily for 2 days at the rate of 37 mg/kg body wt, while the other four sheep received a single oral dose of 400 mg/kg body wt. Preparation of the compound and dosing procedures are described by Tunks *et al.* (1980).

Experiment 3

Five Merino sheep, consuming a daily ration of 600 g of a pelleted mixture of lucerne hay (3 parts) and oat grain (2 parts), were dosed with cyclophosphamide as described by Reis and Chapman (1974). One sheep received an intravenous injection of 3 mg/kg body wt once daily for 21 days; the other four sheep received single oral doses of 30 mg/kg body wt.

Experiment 4

Commencing when they were 6 days old with an average weight of 4 g, 40 mice were injected subcutaneously once daily for 3 days with a solution of L-mimosine as described by Panaretto *et al.*

(1978); the total dose of mimosine averaged 2·7 mg per mouse. Seven days after the first injection of mimosine, 20 treated mice showing at least 90% depilation were selected. At the same time the hair was clipped from the trunk of 20 control mice of the same age, and discarded. Thirteen days later, i.e. 20 days after the first injection of mimosine, regrowth of hair was well established on the mimosine-treated mice. All mice were clipped to give two bulk samples of hair regrown after depilation with mimosine, and hair grown during the same period on control mice.

Experiment 5

(*a*) An area of skin (*c.* 10 by 18 cm) on the midside of a Dorset Horn sheep was closely clipped; the 3-cm length of wool collected served as the control sample. Araldite was applied to the skin, and after hardening overnight, the strip was pulled from the skin. This procedure plucks most fibres from the follicle to the depth of the bulb (Wilkinson 1970). Regrowth samples of wool were harvested by clipping 15, 28, and 42 days after plucking. The regrowth after 15 days was sparse and variable in length, and it appeared that some fibres had merely been broken during plucking and had continued to grow.

(*b*) A complete staple of the birthcoat wool was removed from the midside of a Merino lamb at about 5 months of age. The staple was cut into three approximately equal sections, and the tip and base were kept for analysis.

Experiment 6

The hair was clipped from the trunk of two groups of eight sucking mice as follows:

Group 1. At 13 days of age, and again at 27 days of age.

Group 2. At 27 days of age.

Experiment 7

Two Merino sheep received abomasal infusions of amino acid mixtures including or omitting methionine in successive 12-day periods as described in experiment 3 of Reis and Tunks (1978).

Analytical Procedures

The amino acid composition of wool was determined by hydrolysis *in vacuo* with 6 M HCl containing 2 mM phenol at 108°C for 22 h. After freeze-drying, 1 ml of 0·1 M borate was added and the solution was exposed to the air for 2 h to oxidise cysteine produced by reaction with phenol. The solution was freeze-dried again and its content of amino acids was estimated uing a Beckman Spinco 120C amino acid analyser. Tyrosine was occasionally estimated by a direct assay using the procedure of Bernhart (1938) as modified by Crewther and Dowling (1960). The concentration in wool and hair of the two classes of matrix proteins (high-sulfur group and high-tyrosine group) were calculated from the cystine and tyrosine contents using the appropriate regression curves (Broad *et al.* 1970; Frenkel *et al.* 1974; Gillespie and Frenkel 1974).

The complete amino acid analyses can also be used to provide information on changes in the low-sulfur proteins (using the concentration of leucine and glutamic acid) and the relative proportions of individual components within the high-sulfur and high-tyrosine protein groups.

Results

Composition of Wool Grown under Standard Conditions

In order to determine the extent of variation in the composition of wool grown by a sheep kept under standard conditions, a Merino sheep was housed indoors for about 40 weeks and was given a daily ration of 600 g of a pelleted mixture of lucerne hay (3 parts) and oat grain (2 parts). A staple of wool was collected from the midside of the sheep and was sliced transversely into eight sections, each representing about 5 weeks' growth. Each sample was hydrolysed and its content of amino acids estimated. The proportions of high-sulfur and high-tyrosine proteins, calculated

from the half-cystine and tyrosine contents respectively and expressed as grams per 100 g wool, are shown in the following tabulation:

Protein	Concentration in wool at successive intervals of 5 weeks								
	1	2	3	4	5	6	7	8	Mean
High-sulfur	24·0	23·6	23·8	25·0	24·3	24·6	25·1	24·3	24·3±0·77%, s.d. 0·54
High-tyrosine	9·05	9·33	9·21	8·98	8·59	8·54	8·70	8·65	8·88±0·45%, s.d. 0·30

It can be seen that there is no trend in composition with time and that, within experimental error, the proportions of these two groups of proteins in wool remain constant. Differences that could be regarded as significant (three standard deviations) correspond to about 1·5% high-sulfur protein and 1% high-tyrosine protein.

Changes in the Composition of Wool Grown Following the Administration of Various Chemicals (Experiments 1–3)

Mimosine

Samples of wool were collected from four sheep before they were infused with sufficient mimosine to stop wool growth completely. Wool growth resumed about 2 weeks after the start of infusion and post-treatment samples were collected from wool grown between 3 and 5 weeks (first regrowth sample), 5 to 7 weeks and 9 to 11 weeks after infusion started. The changes in the amino acid composition of wool samples from all four sheep were similar to those shown in Table 2.

Table 1. Protein composition of wool grown before and after intravenous infusion of mimosine (Experiment 1)

Mimosine (120 mg/kg i.v.) was administered to four sheep daily for 2 days. The protein composition of wool samples was determined before infusion and 3–5 and 9–11 weeks after treatment. Values are given as grams per 100 g wool

Sheep No.	Protein	Concentration in wool		
		Pretreatment	At 3–5 weeks	At 9–11 weeks
P8642	High-sulfur	19	24	23
	High-tyrosine	10	6	10
P8690	High-sulfur	22	27	22
	High-tyrosine	10	5	11
P8696	High-sulfur	20	24	21
	High-tyrosine	12	6	11
P8706	High-sulfur	21	27	20
	High-tyrosine	11	5	11
Mean	High-sulfur	21	26	22
	High-tyrosine	11	6	11

Compared with pre-treatment levels, the first regrowth sample from sheep P8696 showed a substantial reduction in tyrosine and glycine content, consequent on a drop in the high-tyrosine protein content from 12% in the control period to 6% in the immediate post-mimosine period. In the 5–7-week sample the level of this protein increased to 10%, but it was only in the 9–11-week period that the high-tyrosine protein content returned to the pre-infusion value. The first regrowth sample also showed a significant increase in the content of cystine and proline indicating that the high-sulfur protein had increased from 20 to 24%. When wool samples from the four sheep are considered (Table 1), the pretreatment wool contained on average 11%

high-tyrosine protein, which dropped to 6% in the period 3–5 weeks after treatment but which returned to the pretreatment levels by 9–11 weeks after treatment. The pretreatment wool on average contained 21% high-sulfur protein. This increased to 26% in the 3–5-week post-treatment samples and returned to a normal level (22%) in the 9–11-week samples. These changes in the relative proportions of the matrix proteins in the first wool regrown after defleecing with mimosine are similar to those observed in wool grown during the continuous infusion of mimosine at lower dosage rates (Frenkel *et al.* 1975).

N-[5-(4-*Aminophenoxy*)*pentyl*]*phthalimide*

Five Merino sheep were treated with *N*-[5-(4-aminophenoxy)pentyl]phthalimide; one sheep (P5033) was given an intravenous infusion for 2 days and the other four sheep were given single oral doses. Wool samples were collected from the five sheep prior to treatment and at intervals up to 11 weeks after dosing. Analytical values for wool samples from one of the sheep (P5144) given an oral dose are shown in Table 2. It can be seen that, following defleecing with this compound, the regrowth wool showed a decrease in tyrosine, glycine and phenylalanine contents and thus in

Table 2. Amino acid and protein composition of wool grown before and after administration of a single oral dose of *N*-[5-(4-aminophenoxy)pentyl]phthalimide (Experiment 2)

Sheep P5144 received a single oral dose (400 mg/kg) of *N*-[5-(4-aminophenoxy)pentyl]phthalimide. Wool was collected and analysed before treatment and 3–5 and 9–11 weeks after treatment. Amino acid content is expressed as residues per 100 residues, and protein content as grams per 100 g wool

Amino acid	Concentration in wool			Amino acid	Concentration in wool		
	Pretreatment	At 3–5 weeks	At 9–11 weeks		Pretreatment	At 3–5 weeks	At 9–11 weeks
Lysine	2·83	2·79	2·91	Valine	5·37	5·55	5·54
Histidine	0·85	0·93	0·84	Methionine	0·40	0·40	0·44
Arginine	6·76	6·80	6·76	Isoleucine	2·86	3·06	3·10
Aspartic acid	5·93	5·65	6·48	Leucine	7·28	6·65	7·76
Threonine	6·10	6·86	6·11	Tyrosine	4·46	2·85	3·96
Serine	11·00	11·30	10·60	Phenylalanine	2·69	2·10	2·81
Glutamic acid	10·90	11·10	11·50				
Proline	6·40	8·06	6·59	High-tyrosine protein	13	4	10
Glycine	9·52	7·07	8·69				
Alanine	5·10	5·10	5·38	High-sulfur protein	26	33	23
Half-cystine	11·50	13·80	10·60				

high-tyrosine protein, together with a large increase (27%) in high-sulfur proteins as determined from changes in the cystine and proline contents. Even after 11 weeks the matrix protein content of the wool had not returned to pretreatment levels for this sheep. Taking the combined data from the four sheep given oral doses (Table 3), the pretreatment wool had on average a high-tyrosine protein content of 12% which decreased in the first regrowth wool to 5% but which essentially returned to pretreatment levels in the 9–11-week samples. The corresponding values for mean high-sulfur protein contents were 23, 28 and 21%.

There were considerable variations both in the pretreatment levels of high-sulfur proteins in wool from the four sheep and in the level to which these proteins were

raised in the first regrowth samples. The largest proportional increases in high-sulfur proteins in the regrowth wool were observed in those sheep (P5144 and P7098) which already had a comparatively high content of these proteins in their wool before dosing. The sheep given an intravenous dose of this chemical showed even larger effects (Table 4) which appeared to be more prolonged than with the sheep dosed orally.

Table 3. Protein composition of wool grown before and after administration of a single oral dose of *N*-[5-(4-aminophenoxy)pentyl]phthalimide (Experiment 2)

Four sheep received a single oral dose (400 mg/kg) of *N*-[5-(4-aminophenoxy)pentyl] phthalimide. Wool samples were analysed before administration and 3–5 and 9–11 weeks after commencement of treatment. Values are given as grams per 100 g wool

Sheep No.	Protein	Concentration in wool		
		Pretreatment	At 3–5 weeks	At 9–11 weeks
P4479	High-sulfur	21	24	19
	High-tyrosine	11	5	11
P5144	High-sulfur	26	33	23
	High-tyrosine	13	4	10
P7081	High-sulfur	19	23	17
	High-tyrosine	13	6	13
P7098	High-sulfur	24	33	24
	High-tyrosine	12	5	10
Mean	High-sulfur	23	28	21
	High-tyrosine	12	5	11

Cyclophosphamide

Daily intravenous doses of cyclophosphamide (3 mg/kg) given to one sheep decreased the rate of wool growth by 75% and resulted in a break in the wool. Wool grown during the last 2 weeks of treatment, allowing for emergence time of the wool, was collected and analysed. The high-tyrosine protein content of this wool was 5% compared with 8% found in the pretreatment control sample. There was a return to normal values within a few weeks after the cessation of treatment.

Table 4. Protein composition of wool grown before and after intravenous infusion of *N*-[5-(4-aminophenoxy)pentyl]phthalimide (Experiment 2)

N-[5-(4-Aminophenoxy)pentyl]phthalimide was intravenously infused into sheep P5033 at a rate of 37 mg/kg for 2 days. Protein composition of the wool was determined before treatment and 3–5, 5–7 and 7–9 weeks after commencement of infusion. Values are given as grams per 100 g wool

Protein	Concentration in wool			
	Pretreatment	At 3–5 weeks	At 5–7 weeks	At 7–9 weeks
High-sulfur	26	37	27	23
High-tyrosine	11	3	5	8

Four sheep were defleeced with single oral doses of cyclophosphamide and the amino acid and protein compositions of the regrowth wool were followed for 13 weeks after treatment. Overall changes in amino acid composition are not presented in this paper but were very similar to those shown in Table 2. On average (Table 5),

the high-tyrosine protein content of the wool dropped from 12% in the pretreatment samples to 4% in the first samples of regrowth wool. Later samples showed increasing levels of these proteins with normal levels being achieved by 9–11 weeks after dosing. In contrast, the content of high-sulfur proteins first increased from 27% in the pretreatment wool to 32% in the first regrowth samples and then fell below normal, reaching a plateau of 23–24% at 7–9 weeks after dosing. Even after 13 weeks, wool was still being produced with a high-sulfur protein content significantly below the pretreatment level. Sheep A2435 was an exception as the content of high-sulfur proteins in its wool rapidly returned to the pretreatment values.

Table 5. Protein composition of wool grown before and after defleecing with cyclophosphamide (Experiment 3)

Four Merino sheep were defleeced with a single oral dose of cyclophosphamide (30 mg/kg). Samples of wool were analysed before defleecing and 3–5, 5–7, 7–9, 9–11 and 11–13 weeks after commencement of treatment. Values are given as grams per 100 g wool

Sheep No.	Protein	Concentration in wool					
		Pretreatment	At 3–5 weeks	At 5–7 weeks	At 7–9 weeks	At 9–11 weeks	At 11–13 weeks
A2426	High-sulfur	26	31	22	22	21	21
	High-tyrosine	12	3	7	10	12	13
A2431	High-sulfur	29	32	27	25	24	25
	High-tyrosine	11	4	7	9	10	11
A2435	High-sulfur	25	34	26	25	25	26
	High-tyrosine	11	3	7	8	9	9
A2443	High-sulfur	27	31	25	24	23	22
	High-tyrosine	13	5	8	10	11	13
Mean	High-sulfur	27	32	25	24	23	24
	High-tyrosine	12	4	7	9	11	12

Changes in the Composition of Hair Following Dehairing of Mice with Mimosine (Experiment 4)

When mice in their first hair-growth cycle were dehaired with mimosine the first regrowth of hair as compared with a control sample had a markedly reduced content of tyrosine, phenylalanine and glycine, corresponding to a 20% decrease in the level of high-tyrosine protein (Table 6). There were also increased levels of cystine and proline, equivalent to a 21% increase in high-sulfur protein. The mouse therefore responds in the same way as the sheep after depilation with mimosine, although, because of the hair-growth cycle in the mouse, long-term changes in the composition of the hair could not be reliably followed.

Effect of Time after Commencement of Follicle Activity on the Composition of Wool and Hair (Experiments 5 and 6)

The reduced content of high-tyrosine proteins in fibres grown immediately after depilation, induced by the various treatments described previously, may be due to differences in the protein composition of fibres normally synthesized and of those grown immediately after a wool follicle commences its long cycle of synthetic activity. To test this hypothesis we examined the effect of time of synthesis on fibre composition in relation to the age of the follicle.

An attempt was made to obtain a group of regenerating follicles by plucking an area of sheep skin (Experiment 5*a*). The preplucking control and the regrowth samples were analysed for their content of high-tyrosine proteins and the results are shown below.

Period of growth after plucking	High-tyrosine protein content (%)	Period of growth after plucking	High-tyrosine protein content (%)
Preplucking	7	15–28 days[A]	3
0–15 days	5	28–42 days	7

[A] This sample represents the first uncontaminated regrowth sample.

The early regrowth samples had a reduced content of high-tyrosine proteins especially in the 15–28-day period after plucking when the content was reduced to about 40% of the control value. However, the wool grown during the 28–42-day period had returned to a normal level.

Table 6. Amino acid and protein composition of mouse hair before and after dehairing with subcutaneous injections of mimosine (Experiment 4)

The concentration of amino acids is expressed as residues per 100 residues and of proteins as grams per 100 g hair

Amino acid	Concentration in hair		Amino acid	Concentration in hair	
	Pre-mimosine	Post-mimosine		Pre-mimosine	Post-mimosine
Lysine	3·04	3·07	Valine	4·24	4·33
Histidine	1·03	0·96	Methionine	0·73	0·68
Arginine	5·61	6·16	Isoleucine	2·39	2·53
Aspartic acid	5·48	5·14	Leucine	6·39	5·80
Threonine	4·76	5·12	Tyrosine	5·01	4·39
Serine	9·61	10·10	Phenylalanine	2·70	2·19
Glutamic acid	12·40	11·30			
Proline	6·36	7·22	High-tyrosine		
Glycine	11·40	10·40	protein	15	12
Alanine	4·34	4·26	High-sulfur		
Half-cystine	13·70	16·00	protein	29	35

High-tyrosine proteins were measured in the tip of a lamb's wool staple corresponding to the first product of follicle activity, and in a base region corresponding to later growth (Experiment 5*b*). The wool tip had a high-tyrosine protein content of 11%, significantly less than that grown later (14%).

A similar experiment was carried out with mice (Experiment 6). In group 1, the first grown hair (0–13 days) contained less high-tyrosine protein than the hair grown between 13 and 27 days (14 and 17% respectively). The content of group 2 hair was 16%, close to the mean of the two group 1 samples suggesting that clipping *per se* did not affect composition. The high-sulfur protein content did not change over the experimental period.

Effects of Infusion of an Imbalanced Mixture of Amino Acids on Synthesis of Matrix Proteins in Sheep (Experiment 7)

Although previous studies (Frenkel *et al.* 1975) have shown that various treatments which weaken wool also cause a partial suppression of the high-tyrosine proteins, it has not been proven that this inhibition is necessary to obtain weakened wool.

Certainly the reverse is not true because high-tyrosine proteins can be suppressed by administration of a mixture of amino acids lacking phenylalanine (Frenkel *et al.* 1974), without reducing the strength of wool.

In recent studies with sheep, Reis and Tunks (1978) have shown that abomasal infusion of a mixture of amino acids lacking methionine results in the growth of weakened wool. The composition of wool from these sheep was examined in order to obtain further information on the possible relationship between wool weakening and the inhibition of high-tyrosine proteins.

Samples of wool grown during the abomasal infusion of amino acid mixtures were collected and analysed; the results are given in Table 7. Although the effects of the supplementation were not the same for both sheep, two results are quite clear. Firstly, in comparing the complete amino acid supplement (which did not weaken wool) with the amino acid mixture lacking methionine (which did weaken wool), the high-tyrosine protein content was either unchanged (sheep 1562) or actually increased (sheep 9053). In no case did it decrease as might have been expected from results with other defleecing agents. Secondly, the complete amino acid supplement caused major increases in high-sulfur proteins in both sheep as might have been expected from previous studies on sulfur-enriched wool. It is of interest to note, therefore, that changes in high-sulfur protein content are not necessarily accompanied by changes in high-tyrosine protein content in spite of the fairly consistent inverse relationship found in this and other studies (Frenkel *et al.* 1974, 1975).

Table 7. Effect of supplementation with amino acid mixtures with and without methionine on the proportions of matrix proteins in wool (Experiment 7)

Sheep No.	Treatment	Protein content (%)	
		High-sulfur	High-tyrosine
1562	Control	19	10
	Complete mixture	29	7
	No methionine	23	8
9053[A]	Control	24	11
	Complete mixture	32	8
	No methionine	21	11

[A] With sheep 9053, the 'no methionine' supplement was given before the complete mixture.

Discussion

It is possible that some of the variations in the composition of wool, which are attributed in this paper to the effects or after-effects of dosing with different chemicals, are naturally occurring. However, Reis (1965) has shown that the sulfur content, and hence the high-sulfur protein content, of wool grown by Merino and crossbred sheep remains relatively constant when sheep are given a standard ration. Moreover, sulfur levels in wool are reproducible when sheep are returned to the same ration after nutritional treatments that increase the sulfur content of wool (Reis 1965, 1967). Further evidence for the constancy of the composition of wool grown under standard conditions, with respect to both high-sulfur and high-tyrosine proteins, has been presented in this paper. It is probable that the small variations which were observed under standard conditions were due to the combined analytical errors of the protein

hydrolysis and amino acid analysis procedures, and it can be concluded that the changes observed in the composition of wool following treatment with various chemicals are, in fact, real.

It is unlikely that the long-term suppression of the high-tyrosine proteins in the regrowth of wool after defleecing with various chemicals is due to the chemicals *per se* because these chemicals are rapidly removed from the body. With mimosine, 88% is excreted within 4 days, either as unchanged mimosine or mimosinamine, and most of the remainder is excreted within the next few days (Hegarty *et al.* 1964; Reis *et al.* 1975). Likewise there is evidence that *N*-[5-(4-aminophenoxy)pentyl]-phthalimide is eliminated fairly rapidly from the body. Following oral doses of 12 g of ^{14}C-labelled compound, 76% of the ^{14}C is excreted in 3 days and 92% in 8 days (A. M. Downes, personal communication). Cyclophosphamide and various biologically active metabolites are eliminated rapidly in the urine of sheep. A large proportion of an oral dose of cyclophosphamide (30 mg/kg body wt) is excreted within 24 h of dosing, and elimination of active metabolites is virtually complete within 5 days (Bakke *et al.* 1972; Schaumlöffel *et al.* 1973). Nevertheless, the possibility of small amounts of active residues of these compounds remaining in the body cannot be completely discounted. The experimental evidence with mimosine does not, however, support such a possibility because Ward and Harris (1976) showed that DNA synthesis quickly recovers after removal of mimosine from follicle bulb cells, i.e. there is no long-term suppression of cell division or protein synthesis. Moreover, in contrast to the effects of continuous mimosine infusion (Reis 1975), the rate of wool growth is actually enhanced following defleecing with mimosine (Reis 1975, 1978; Reis *et al.* 1978) and this effect is still evident after 10 weeks.

Defleecing agents, depending on their chemical nature and dose level, may cause a substantial decrease in the rate of wool growth and result in the production of weakened wool. When this condition is induced by mimosine or cyclophosphamide given at low dose rates there is also a short-term partial suppression of the high-tyrosine proteins. These chemicals may also cause the temporary cessation of wool growth and allow the removal of the fleece. After a variable period of time the follicle recommences synthetic activity. Following treatment with mimosine and cyclophosphamide, initial regrowth is seen 17–18 days after commencement of infusion but 30–50% of the follicles are still inactive after 3 weeks and a few percent after 3–5 weeks (Reis and Chapman 1974). It is this complete cessation of wool growth which seems an essential prerequisite for the long-term suppression of the high-tyrosine proteins.

It was considered possible that the mechanism of this apparent long-term inhibition of high-tyrosine protein synthesis was not the direct consequence of treatment with a defleecing agent but rather an indirect result of the difference in composition between the first regrowth of wool and wool grown before defleecing. In order to examine this proposition we have tried to simulate the regrowth situation in two ways. Firstly, by examining the composition of newly grown wool following plucking, and secondly by looking at the composition of the first hair grown by the follicles of young sheep and mice. The results of these experiments indicate that the first proteins synthesized by a new or regenerating follicle are lower in high-tyrosine proteins than those synthesized later. However, none of these treatments can reproduce exactly the situation of a follicle switched off by a defleecing agent and then regenerating. Consequently the results may be regarded at this stage as merely suggestive that the inhibition of

the synthesis of the high-tyrosine proteins during production of weakened wool and in the early regrowth after defleecing are due to two different phenomena.

In previous studies it was found that weakened wool produced during various treatments was lower in high-tyrosine protein than normal, and it was suggested that this inhibition may be an essential part of the wool-weakening process (Frenkel *et al.* 1975). However, two observations made in the present study do not support this suggestion. Firstly, the treatment of sheep with a supplement of a mixture of amino acids lacking methionine weakens wool without a concomitant suppression of the high-tyrosine proteins. Secondly, regrowth wool, although low in high-tyrosine proteins, is of normal strength. It is clear, therefore, that a reduction in high-tyrosine proteins is not a necessary prerequisite for the production of weak wool. However, this conclusion may be invalid if there is more than one mechanism for the production of weak wool.

The changes observed in the high-sulfur proteins in regrowth wool probably stem from several causes. It is possible that the above-normal high-sulfur protein content of the first regrowth sample is a characteristic of the first wool grown. Analyses of birthcoat samples of lamb's wool support this suggestion (Reis 1970). The subsequent fall to below-normal values is probably at least in part of dietary origin caused by the enhanced rate of wool regrowth (Reis 1975) which, at a constant level of sulfur–amino acid intake, will result in the lowering of the high-sulfur protein content of wool produced during this period (Gillespie and Reis 1966). These long-term changes in composition are accompanied by alterations in the relative proportions of individual high-sulfur and high-tyrosine protein components (R. C. Marshall, personal communication).

The consequences of these long-term changes in the composition of wool on its chemical and physical properties are not known. However, the relationship found for other keratins between mechanical properties and their matrix content (Bendit and Gillespie 1978) suggests that wool grown by a sheep after a defleecing episode may have temporarily changed mechanical or chemical properties, or both, to which must be added crimp and diameter, which will all vary along the length of the fibre. Work should be done to establish the magnitude of these changes.

It has been pointed out to us that as the variations in composition along the length of the fibre are encompassed by the maximum variations in composition found for wool samples from individual sheep within a flock (Frenkel *et al.* 1974) they may be of little significance when the wool is converted to textiles. This argument ignores the fact that the variability following chemical defleecing will be added to the natural intraflock variablity and in any case variability along the length of a fibre introduces an entirely new dimension. Resolution of this problem must await the results of textile processing.

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References

Bakke, J. E., Feil, V. J., Fjelstul, C. E., and Thacker, E. J. (1972). Metabolism of cyclophosphamide by sheep. *J. Agric. Food Chem.* **20**, 384–8.

Bendit, E. G., and Gillespie, J. M. (1978). The probable role and location of high-glycine-tyrosine proteins in the structure of keratins. *Biopolymers* **17**, 2743–5.

Bernhart, F. W. (1938). Microdetermination of the tyrosine content of protein hydrolysates. *J. Biol. Chem.* **123**, x.

Broad, A., Gillespie, J. M., and Reis, P. J. (1970). The influence of sulphur-containing amino acids on the biosynthesis of high-sulphur wool proteins. *Aust. J. Biol. Sci.* **23**, 149–64.

Crewther, W. G., and Dowling, L. M. (1960). The action of nitric and hydrochloric acids on wool with particular reference to supercontraction of the fibers. *Text. Res. J.* **30**, 23–36.

Crounse, R. G., Maxwell, J. D., and Blank, H. (1962). Inhibition of growth of hair by mimosine. *Nature* (*London*) **194**, 694–5.

Frenkel, M. J., Gillespie, J. M., and Reis, P. J. (1974). Factors influencing the biosynthesis of the tyrosine-rich proteins of wool. *Aust. J. Biol. Sci.* **27**, 31–8.

Frenkel, M. J., Gillespie, J. M., and Reis, P. J. (1975). Studies on the inhibition of synthesis of the tyrosine-rich proteins of wool. *Aust. J. Biol. Sci.* **28**, 331–8.

Gillespie, J. M., and Frenkel, M. J. (1974). The diversity of keratins. *Comp. Biochem. Physiol.* **47B**, 339–46.

Gillespie, J. M., and Reis, P. J. (1966). The dietary-regulated biosynthesis of high-sulphur wool proteins. *Biochem. J.* **98**, 669–77.

Hegarty, M. P., Schinckel, P. G., and Court, R. D. (1964). Reaction of sheep to the consumption of *Leucaena glauca* Benth. and to its toxic principle mimosine. *Aust. J. Agric. Res.* **15**, 153–67.

Montagna, W., and Yun, J. S. (1963). The effects of the seeds of *Leucaena glauca* on the hair follicles of the mouse. *J. Invest. Dermatol.* **40**, 325–32.

Panaretto, B. A., Tunks, D. A., and Munro, S. (1978). Depilatory effects of certain chemicals during the first hair growth cycle in sucking mice. *Lab. Anim.* **12**, 185–92.

Reis, P. J. (1965). Variations in the sulphur content of wool. In 'Biology of the Skin and Hair Growth'. (Eds A. G. Lyne and B. F. Short.) pp. 365–75. (Angus and Robertson: Sydney.)

Reis, P. J. (1967). The growth and composition of wool. IV. The differential response of growth and of sulphur content of wool to the level of sulphur-containing amino acids given per abomasum. *Aust. J. Biol. Sci.* **20**, 809–25.

Reis, P. J. (1970). The influence of dietary protein and methionine on the sulphur content and growth rate of wool in milk-fed lambs. *Aust. J. Biol. Sci.* **23**, 193–200.

Reis, P. J. (1975). Effects of intravenous infusion of mimosine on wool growth of Merino sheep. *Aust. J. Biol. Sci.* **28**, 483–93.

Reis, P. J. (1978). Effectiveness of intravenous and abomasal doses of mimosine for defleecing sheep and effects on subsequent wool growth. *Aust. J. Agric. Res.* **29**, 1043–55.

Reis, P. J., and Chapman, R. E. (1974). Changes in wool growth and the skin of Merino sheep following administration of cyclophosphamide. *Aust. J. Agric. Res.* **25**, 931–43.

Reis, P. J., and Panaretto, B. A. (1979). Chemical defleecing as a method of harvesting wool from sheep. *World Anim. Rev.* **30**, 36–42.

Reis, P. J., and Tunks, D. A. (1978). Effects on wool growth of the infusion of mixtures of amino acids into the abomasum of sheep. *J. Agric. Sci.* **90**, 173–83.

Reis, P. J., Tunks, D. A., and Downes, A. M. (1978). Mimosine, administered orally, and two related compounds as chemical defleecing agents for sheep. *Aust. J. Agric. Res.* **29**, 1065–75.

Reis, P. J., Tunks, D. A., and Hegarty, M. P. (1975). Fate of mimosine administered orally to sheep and its effectiveness as a defleecing agent. *Aust. J. Biol. Sci.* **28**, 495–501.

Schaumlöffel, E., Habermehl, A., Brock, N., and Schneider, B. (1973). Studies on the pharmacokinetics of cyclophosphamide in sheep. *Arzneim. Forsch.* **23**, 491–500.

Tunks, D. A., Rigby, R. D. G., Downes, A. M., Lamberton, J. A., Panaretto, B. A., and Reis, P. J. (1980). *N*-[5-(4-Aminophenoxy)pentyl]phthalimide as a potential chemical defleecing agent and its effects on wool growth. *Aust. J. Agric. Res.* **31**, in press.

Ward, K. A., and Harris, R. L. N. (1976). Inhibition of wool follicle DNA synthesis by mimosine and related 4(1*H*)-pyridones. *Aust. J. Biol. Sci.* **29**, 189–96.

Wilkinson, B. R. (1970). Keratin biosynthesis. I. Isolation and characterization of polysomes from wool roots. *Aust. J. Biol. Sci.* **23**, 127–38.

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