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Preliminary Observations on Chemical Defleecing of Sheep as a Means of Reducing Carcass Contamination

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Introduction

The difficulties of producing meat of good hygienic standard and satisfactory keeping quality from dirty animals are well recognised. Investigations carried out for Australian and New Zealand meat exporters, quoted by Scarafondi (1957), showed that about a third of the superficial contamination occurring on carcases was derived from dirt introduced into the slaughterhall with the live animal. Control of this source of contamination is particularly difficult with sheep, the often dirty fleeces of which readily contaminate abattoir equipment and slaughtermen's hands and arms. Washing animals before slaughter can reduce the weight of contamination in warm, dry climates, but it is of doubtful value in regions with high relative humidity, as the fleece becomes sodden and spread of contamination at slaughter may even be exacerbated. Shearing before slaughter also reduces contamination, but requires skilled labour and so is expensive. Attention therefore needs to be given to other means of combatting this problem.

The occurrence of alopecia as a characteristic toxic effect in the clinical administration of certain antitumour agents led Homan, Mendzian and Busey (1968) to postulate that, if the chemical compound concerned acted primarily on dividing cell, hair loss should most readily be observed in animals with continuous hair growth. They showed that a single intravenous dose of 30 to 40 mg per kg body weight of cyclophosphamide, the inactive "transport form" of cytostatic and alkylating agents of the Nitrogen Mustard series (Fishbein and Falk, 1969), resulted in complete hair loss in a number of species, including Suffolk sheep. Following up this finding, Dolnick, Lindahl, Terrill and Reynolds (1969) studied the possibility of using cyclophosphamide for defleecing sheep, with the objective of avoiding the heavy cost of shearing in the United States, due to the shortage of skilled shearers and consequent high wages. They found that the fleece could be removed manually about seven days after treatment with a single dose of 10 to 30 mg of the drug per kg body weight. The sheep was left bare and they recommended delaying removal of the fleece until at least three weeks after dosing, so that a short new growth remained to protect the sheep when the fleece was pulled off.

When defleecing for hygienic reasons, sheep could have their fleeces removed immediately before slaughter, as there would be no need to allow time for the new growth, below the constriction of fibre caused by cyclophosphamide, to extend above the level of the skin.

Materials and Methods

Sheep. The sheep used in these experiments were shearing ewes and wethers of various crosses of the Clun Forest, Colbred, Devon Longwool, Dorset, Suffolk and other Downs, Isle de France, Finn and Kerry Hill breeds. At the time of dosing, the mean weight of the thirty sheep in Trials 1 to 8 (see Table 1) was 42.4 kg (S.D. 7.09). Thirty-seven older sheep were defleeced in Trial 9, their mean weight was 65.5 kg (S.D. 12.82).

These sheep were kept at grass, receiving a daily supplement of ½ lb proprietary concentrate per head. They were brought into the lairage, with the controls, on the evening before defleecing and slaughter. The sheep which received toxic doses of cyclophosphamide were kept penned until slaughter or death.

Drug Administration. (a) defleecing
Cyclophosphamide was made up in a 1% w/v aqueous solution and given as a drench at doses ranging between 20 and 30 mg per kg body weight.

(b) toxicity trials
A 4.5% w/v aqueous solution of the drug was injected subcutaneously into the flanks, the volume being divided between both sides, at a dose rate of 90 mg per kg body weight.

Microbiology. (a) carcase contamination
The entire inside and outside surface of each carcase was individually swabbed. The swabs were then thoroughly washed in maintenance medium, decimal dilutions of which were sown onto plates of tryptone glucose yeast extract agar (Oxoid CM 325), with 1% sodium chloride, and incubated at 25°C for four days. Counts were then made and expressed as millions of organisms per carcase.

(b) blood examination
Blood was drawn from the jugular vein, using a sterile hypodermic syringe rinsed with heparin solution. 2 ml was transferred to a sterile Universal container holding 0.5 ml of 1% sodium citrate solution. In the laboratory the blood was mixed with 20 ml digest agar at 45°C and plated. Plates were incubated at 37°C for 48 hours, being examined after 24 hours and 48 hours.

Biopsy. A strip of fleece, running from the shoulders to the rump centred on the mid line of the back and about 8 in. wide, was close clipped. Immediately before sampling, the areawas throughly cleaned with industrial spirit. Biopsy was carried out by the high speed punch technique of Findlay and McEwan (1960). A punch of 5 mm internal diameter produced a sample too small for accurate alignment when cutting sections; a punch with an internal diameter of 10 mm was made and this proved satisfactory. The resulting wound was packed with 0.5% w/w centrimide cream, B.P., and no trouble was experienced with infection. Biopsy samples were at once transferred to 10% formol saline and subsequently sectioned at 5μ and 10μ, before staining by blue trichrome, van Gieson or haematoxylin and eosin.

Blood cell counts. Blood was drawn from the jugular vein by hypodermic syringe, into a Bijou bottle containing E.D.T.A. as an anticoagulant. It was throughly mixed and differential white cell counts were made in an automatic counter.

Tissue residues. The quantitative estimation of the active allylating products of cyclophosphamide after metabolism was undertaken by the technique of Epstein, Rosenthal and Ess (1955), modified to a colorimetric method (Brock and Hohorst, 1967). Tissue samples were held at -20°C if they were not to be examined immediately.

25 ml heparinised blood was centrifuged at 4,000 r.p.m. for 25 minutes and 10-15 ml plasma was then taken for examination; 30g samples of liver, kidney, abdominal fat and muscle were added to 90 ml of 0.1 M potassium chloride and homogenised in a Waring blender. 1 ml perchloric acid was then added to 1 ml of plasma, tissue homogenate, bile or urine and boiled for twenty minutes, after which the pH was adjusted to 4.6 with 2 ml of 0.2 M acetate buffer (1.4 vol. 0.2 M acetate buffer pH 4.6 and 0.6 vol. 1 M sodium hydroxide) and 0.5 ml 5% 4 - (4 nitrobenzyl) pyridine was also added. The mixture was held in boiling water for twenty minutes and then put into crushed ice. 2 ml acetone, 5 ml ethyl acetate and 2 ml 1 M sodium hydroxide were added, the mixture was shaken vigorously twenty times and centrifuged at 2,000 r.p.m. for 30 seconds. The optical density of the organic phase was then measured at 540 in a Unicam SP800 spectrophotometer.

Redistilled ethylene dichloride was sometimes used in place of ethyl acetate. In these cases, the mixture was stood in crushed ice for 30 minutes and then centrifuged for 5 minutes at 2,000 r.p.m. before the optical density was measured. The ethylene dichloride phase must be covered by a layer of 1 M sodium hydroxide solution to prevent rapid decay of colour.

Results

Defleecing and Carcase Contamination. Table 1 gives details of the various groups of sheep which have been defleeced after treatment with cyclophosphamide in the course of these observations:-

Trial No.	Number of sheep treated	Dose mg/kg	Days to defleecing
1	5	20	8
2	6	30	7(3°), 8(2°), 14(1°)
3	6	30	8
4	6	25	10 (2*)
5	3	30	10
6	1	30	9
7	1	25	10 (1*)
8	1	30	9
9	37	30	12 (1*)

* Number of sheep in trial not defleeced at first attempt.
° Number of sheep defleeced on post-treatment day shown.

No clinical signs of toxicity were noted at this dose level. If not defleeced within ten days, some sheep began to lose their wool spontaneously. As shown in the Table, four treated sheep could not be defleeced manually at the first attempt; it is not known whether this was due to individual idiosyncracy or to underdosing, due to spillage when drenching. Three of these sheep were slaughtered at once, but the fourth, in Trial 9, was defleeced satisfactorily on the following day, thirteen days after treatment. The impression has been gained that sheep with some black pigment in their wool are less easily defleeced than sheep of all white varieties.

Table 2 shows the results of investigations on carcase contamination with groups of treated and control sheep in the first five trials.

Trial No.	Number of sheep treated	No. of control sheep	Mean 10^6 organism per carcase		Reduction of bact. load
			Treated	Controls	
1	6	6	15.74	36.81	57%
2	6	6	Not Done		
3	6	6	1.66	5.95	72%
4	4	4	1.03	3.14	67%
5	3	3	4.82	70.47	93%

In the first trial the treated sheep escaped into the pen holding the controls after defleecing. This probably accounts for their relatively high bacterial load compared with the defleeced sheep in the other three trials. Although the sheep in the fifth trial were dirtier than the rest, none of these groups was heavily contaminated and the carcases from the controls generally had a good visual appearance when dressed. With groups of dirtier sheep, more marked reductions in bacterial counts after defleecing might have been achieved.

Hair Follicle and Wool Fibre Examination. Biopsies were taken immediately before dosing with 30 mg cyclophosphamide per kg body weight and 2, 8, 24, 72 and 168 hours afterwards. No significant abnormalities were observed in the sections of samples at two and eight hours after treatment, but the sections from material at 24 hours showed focal degeneration of the follicle germinal cells adjacent to the dermal papilla, with pyknotic debris and cellular contraction. Similar changes were present in the sections of the biopsy collected at 72 hours and very small amounts of pyknotic nuclear debris were occasionally noted among the germinal cells in the 168 hours sample.

Samples of skin and wool from sheep dosed with 30 mg/kg body weight were sent to the laboratories of the Wool Industries Research Association. The skin sample had been taken at slaughter, nine days after treatment, and histological examination showed that the follicles had shed their fibres and were growing new ones. The wool, collected from several sheep, was of normal appearance and handled similarly to that of conventionally sheared sheep. Fibre strength was good, there being no evidence of break in the staple, and the microscopic appearance was typical of shed fibres. Examination of stained fibres showed the majority to be fully keratinised; the very small number of unkeratinised fibres suggested that shedding took place in the follicle above the keratinisation level.

Bacteriological Blood Counts and Pathological Examination. Ewe hogget 321, weighing 34 kg, was given 90 mg/kg cyclophosphamide. Blood samples were taken from the jugular vein for bacteriological counts before treatment and ½, 1, 2, 3, 4, 5, 6, 7 and 24 hours afterwards; a sample was also collected when the animal was bled out 30 hours after dosing.

After twenty-four hours incubation, single surface colonies were present on the plates made with samples collected 6 and 24 hours after treatment. A few surface colonies were present on plates made from other samples when they were re-examined after forty-eight hours incubation. It is thought these organisms had been introduced when the plates were inspected after twenty-four hours incubation. Blood collected at the time of slaughter was heavily contaminated with many different types of organisms, probably derived from the fleece and skin. These findings suggest that even a heavily toxic dose of cyclophosphamide does not impair the integrity of the gut wall and so enable bacteria from the intestine to enter the circulating blood.

Histological examination of sections from the duodenum, ileum, caecum and rectum tended to confirm this result, as the epithelium was intact. Sections of the liver showed some fatty infiltration. The uro-genital tract was markedly affected; kidney glomeruli were disintegrating and the tubules swollen. The bladder wall was oedematous and much of its epithelial lining was absent.

Toxicity. Four sheep were given 90 mg cyclophosphamide per kg body weight. All these sheep showed depression and ~~incoordination~~. One was killed twenty-four hours after treatment, another after thirty hours, the third at forty-eight hours and the fourth died about 51 hours after dosing.

On post-mortem examination, the urinary tract was inflamed; histological examination showed the kidney glomerule disintegrating, tubules swollen and the bladder wall oedematous, with much of its lining absent.

The total white blood cell (W.B.C.) count fell rapidly after a single dose at this level, the number of lymphocytes in the circulating blood being greatly reduced, which polymorphonuclear leucocytes increased. The results obtained with sheep 135 (38.6 kg live weight), which was slaughtered forty-eight hours after dosing, were typical.

	Total W.B.C.	Lymphocytes	Polymorphs
Pre-treatment	11,900	7,973	2,618
26½ hours post-treatment	7,400	3,034	4,144
48 hours post-treatment	5,540	1,994	3,490

(all figures per cu. mm)

Sheep dosed with 20 to 30 mg cyclophosphamide per kg body weight showed no clinical symptoms or gross lesions on post mortem. However, some degree of leucopenia developed. Table 3 gives the mean daily values obtained from six sheep given 25 mg/kg of the drug and from six controls.

Table 3

		Pre-treatment	Days post-treatment					
			3	4	5	6	7	8
Dosed	Total W.B.C.	11,930	9,480	7,600	7,560	8,200	8,560	8,200
	Lymphocytes	8,897	6,879	5,552	5,804	6,123	6,177	5,582
	Polymorphs	2,208	2,186	1,698	1,474	1,717	1,962	2,433
Controls	Total W.B.C.	11,650	12,360	12,500	12,500	12,700	13,200	10,650
	Lymphocytes	7,389	8,167	8,560	8,127	7,765	9,146	7,335
	Polymorphs	3,317	3,493	3,281	3,482	3,935	3,113	3,059

Tissue Residues. Blood samples were taken immediately before treatment and at intervals thereafter. Due to mechanical failure of the spectrophotometer, no results were obtained from the blood of the sheep killed at twenty-four hours, but the results from the other three are shown in Table 4.

Table 4

Time (hours)	Sheep Number 430 Absorbance		Sheep Number 135 Absorbance		Sheep Number 321 Absorbance	
	Active Alkylating	Total Agents	Active Alkylating	Total Agents	Active Alkylating	Total Agents
¼	0.30	1.44	0.09	1.15	0.08	1.12
1	-	-	-	-	0.22	0.78
2	0.57	0.94	0.38	0.68	0.40	0.64
3	-	-	-	-	0.47	0.56
4	0.61	0.64	0.52	0.54	0.49	0.51
5	-	-	-	-	0.45	0.45
6	-	-	-	-	0.38	0.38
7	-	-	-	-	0.26	0.26
24	0	0	0	0	0	0
30	-	-	-	-	0	0
48	-	-	0	0	Slaughtered	
	Died		Slaughtered			

- Not done

Post-mortem samples of the liver, kidneys, abdominal fat and of the semitendinosus, semimembranosus and longissimus dorsi muscles were collected for examination, as well as specimens of bile and urine. Cyclophosphamide or its metabolites were shown to be present only in the liver of the sheep killed twenty-four hours after dosing, but tests on the material collected from the sheep killed after thirty and forty-eight hours were all negative.

Discussion

The results confirm that the majority of sheep can be defleeced readily from about eight days after treatment with 20 to 30 mg. cyclophosphamide per kg. body weight. The impression has been gained that those breeds of sheep with some black pigment, e.g. Suffolks and their crosses, are less readily defleeced than sheep without pigmented wool. Chemical defleecing immediately before slaughter results in reduction of the bacterial load on carcases from comparatively clean sheep. An even more substantial reduction might be achieved with sheep having dirty fleeces. If the manufacturers of the drug seek and obtain government approval for routine use of cyclophosphamide as a defleecing agent, it is proposed to organise a large scale trial, in conjunction with a commercial abattoir, to evaluate the hygienic benefit of the method under field conditions.

Changes in the germinal cells of the hair follicle soon after dosing with cyclophosphamide cause the fibres to be shed, but fresh growth is resumed shortly afterwards. The quality of the skin and of the fleece are not affected. Hourihan, Derrill and Wilson (1970) state that the marketability of the wool may be improved, both by the greater ratio of top to noils in the fleece and also by the absence of second cuts.

Doses of about 90 mg/kg are severely toxic (Dolnick et al., 1969 and 1970) and have been shown here to cause a marked leucopenia and gross damage to the urinary bladder in sheep; lesions in the mucosa of the urinary bladders of dogs, which had received repeated intravenous doses of cyclophosphamide, were reported by White (1958) and detailed descriptions of these changes in dogs and in rats were given by Philips, Sternberg, Cronin and Vidal (1961), who stated that bladder damage could be prevented by promoting active diuresis during the first few hours after injecting the drug. Nevertheless, even with a 90 mg/kg dose level, no evidence was found of impairment of the gut wall and of invasion of the blood stream by enteric organisms. The doses used in the defleecing trial (20 to 30 mg/kg) caused no clinical symptoms, although some reduction of the white blood cell count occurred. Homan et al (1969) state the values return to normal within seven days, but the results in Table 3 show that pre-treatment values had not been fully recovered eight days after dosing.

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Acknowledgements

I am very grateful to Dr.D.F. Kelly and Mr.A.I. Wright, School of Veterinary Science, Bristol University, for commenting on histological changes in sections of skin biopsy material from sheep dosed with cyclophosphamide and to Mr. A.D. Osborne, also of the veterinary school, for reporting on post-morten sections of organs from sheep which had received toxic doses of cyclophosphamide, as well as arranging for the white cell counts on blood samples from treated and control sheep. Dr. H.M. Appleyard, Wool Industries Research Association, commented on the quality of wool samples and on histological changes in sections of skin from chemically defleeced sheep; in addition, he supplied photomicrographs showing the microscopical changes in these tissues.

I also want to thank the following colleagues in the Meat Research Institute: Mr. W.R. Hudson, for bacteriological examination of carcases from chemically defleeced and control sheep, as well as of blood samples from sheep treated with toxic doses of cyclophosphamide; Miss J.C. Pepper, for the biochemical examination of tissues for residues of the drug and its metabolites; Mr. D.J.Restall for histological sections of biopsy material from chemically defleeced sheep and Mr. R.Warrington, for assistance on many aspects throughout these observations.

The assistance of Asta Werke A.G. and Ward Blenkinsop & Co., Ltd., in supplying cyclophosphamide is gratefully acknowledged.

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