## FURTHER STUDIES OF FLUOROSIS IN MERINO SHEEP.

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## SUMMARY.

Further studies were made on six sheep which had been continuously exposed to fluorided water from 3 months to 27 months of age. They were maintained for a period of 30 months during which they had access to grazing and grassy lucerne hay and were protected from fluorine in the drinking water.

Observations were made on the permanent incisor teeth. Autopsies were carried out at the end of the 30 months on fluorine-free water. X-ray photographs were taken and bones, teeth and some organs were analysed for fluorine. Comparison of these data with those previously reported for similar sheep immediately after the period of exposure to fluorine supports the following conclusions:—

(1.) The effects of fluorosis on the incisor teeth are permanent. The brittleness of affected teeth and the lines of weakness at bands of pitting result in increased rather than decreased damage. Teeth which erupt after 12 months' protection contain less fluorine and are less severely affected, but positional defects at eruption still result in a badly deformed incisor arch.

(2.) Bone defects such as rarification and shortening of the horizontal ramus of the mandible, which were apparent at the end of the period of exposure to fluorine, are not permanent.

### INTRODUCTION.

Fractures and excessive wear of the teeth are constant features of fluorotic Merino sheep that exist solely by grazing natural pasture in the field. This is not true of experimentally induced fluorosis in penned sheep. It has been assumed that the lesions are accentuated in the field by the fibrous food which for several months of each year represents the bulk of the grazing.

Experimental sheep with a known history of fluorosis were available to test the hypothesis. The work was superimposed on a study involving the recuperation of young fluorotic sheep kept on fluorine-free water and the results are now recorded together.

The objects of the investigation were :---

- (1) To study the effects of grazing or simulated grazing on the teeth of sheep already damaged by fluorosis.
- (2) To obtain information on fluorine storage in bones and teeth after 24 months' exposure to and 30 months' protection from water containing excessive fluorine.
- (3) To make further observations on the bone rarification recorded in previous studies.

### J. M. HARVEY.

### EXPERIMENTAL.

The six experimental sheep were bred in south-western Queensland from ewes which had not been exposed to water containing more than 0.5 p.p.m. fluorine. As 3-4-month-old lambs they were transferred to the Animal Health Station, Yeerongpilly, and were used immediately for two years in the experiment previously reported (Harvey, 1952). During that period they were housed in pens and fed cereal chaff plus milled supplements. The drinking water for some contained 10 p.p.m.F and for others 5 p.p.m.F.

For the following 30 months they had access to grazing, mainly a couch (*Cynodon dactylon*) and paspalum (*Paspalum dilatatum*) pasture. Grassy lucerne hay was fed in bales. The sole drinking supply was city water, which did not contain more than 0.2 p.p.m.F.

Observations were made on the incisor teeth at monthly intervals. Photographs of the incisor teeth were taken at 27 months, 51 months and 57 months of age. After autopsy at 57 months of age, X-ray studies were made on femur, tibia and mandible. Fluorine analyses were done on muscle, kidney, femur, tibia, metatarsus, mandible and incisor, molar and premolar teeth.

### RESULTS.

Table 1 records the observations on the incisor teeth of the six experimental animals. Figs. 1-6 show the incisor teeth after 24 months' exposure to fluorided water and after a further 24 months and 30 months, during which they were protected from fluorided water.

Table 2 records the fluorine content of muscle, kidney, bones and teeth from the six sheep. Examination of the analytical data shows the following features:---

- (1) The concentration of fluorine in both kidney and muscle is similar to that recorded at the end of 24 months' continuous exposure to fluorine in the drinking water.
- (2) In all six sheep, there is a marked reduction in the fluorine concentration in femur and mandible when compared with levels recorded in sheep of the same group slaughtered 30 months previously, at the conclusion of the period of exposure to water containing fluorine. This reduction in fluorine concentration is most marked in the mandible. The concentration of fluorine also shows a tendency to fall in the tibia, whereas fluorine levels have remained relatively constant in the metatarsus.
- (3) The fluorine concentration in the incisor teeth does not differ markedly from levels recorded immediately after the exposure period. The levels of fluorine in the 4th pair of incisors are much lower than those found in the 2nd and 3rd pairs. This difference is less marked in sheep F, but the explanation lies in the fact that the 4th pair of incisors in this sheep had erupted

within four months of transfer to fluorine-free water. For sheep A, B, C, D, and E, eruption of the 4th pair of incisors did not occur for 12 months or longer after transfer to fluorine-free water.

(4) The fluorine levels in premolar and molar teeth tend to be higher than the levels recorded previously in similar sheep slaughtered immediately after exposure to fluorine.

X-ray photographs of the femur, tibia and mandible are shown in Figs. 7-18. In none of the sheep is the bone rarification noted previously at 27 months of age apparent at 57 months of age. There has been marked lengthening of the horizontal ramus of the mandible, and the roots of the molar and premolar teeth no longer extend into the compact substance. There is evidence of uneven wear on both molar and premolar teeth in some sheep.

### DISCUSSION.

All the incisor teeth remaining at the conclusion of this investigation show the characteristic lesions associated with fluorosis. These lesions are emphasised by staining due to degradation products of substances present in the diet during the grazing period only. In each of these six sheep the damage from fluorosis has been most marked in the 3rd pair of incisors. The damage to the 4th pair is less severe, but in all sheep one or both of these incisors have erupted at right-angles to the normal plane. A badly deformed incisor arch has resulted in all sheep. The shedding of the 1st pair and in some cases the 2nd and 3rd pair of incisors probably arose from the 24-month period from 3 months to 27 months of age when these sheep were maintained entirely on chaffed or milled feed. In areas of endemic fluorosis in Queensland, although excessive wear and breaking of incisors at lines of pitting have been characteristic, the shedding of incisors has not been noted.

Unevenness of the cutting surfaces of the molars is noticeable in the X-ray photographs from some of these sheep. It is less severe than that noted in specimens from endemic areas. The explanation probably lies in diet. First, these sheep were maintained from the age of 3 months to 27 months on chaffed feed; and secondly, from the age of 27 months to 57 months they were fed pasture and lucerne hay—this would involve less wear on the molar teeth than the harsh stubble-like pasture that is often the sole source of feed for many months of each year in parts of Queensland where fluorosis is endemic.

X-ray examination shows that the bone rarification, noticeable at the conclusion of the 24-month exposure period, is not apparent at the conclusion of the 30-month protection period. For each of these six sheep there has been a considerable thickening of the compact substance of the femur and tibia, and more particularly of the mandible. There has also been a marked lengthening of the horizontal ramus of the mandible. Overcrowding of the molars, which was apparent earlier, and which resulted in irregular cutting surfaces, is no longer noticeable.

Table 1.Comments on Incisor Teeth.

		Comments on Incisor Teeth.						
Sheep.	History from 3 to 27 months of age.	At transfer to grazing. Age 27 months.	After 12 months' grazing. Age 39 months.	After 24 months' grazing. Age 51 months.	At slaughter after 30 months' grazing. Age 57 months.			
A	Group 2A. Ration : oaten chaff + bonemeal. Water : 5 p.p.m.F.	2nd pair in wear; all teeth show erosion, horizontal chalky bands and chip- ing of cutting edges; a badly deformed incisor arch.	3rd pair almost in wear, erupted at right angles, chalky and show erosion emphasised by consider- able staining.	Ist pair, left only has been shed; 2nd pair show increased staining at the base; 3rd pair markedly eroded and stained.	1st pair, both shed; 2nd pair as previously described; 3rd pair as previously described; 4th pair almost in wear, erupted at right angles, stained but not markedly eroded.			
В	Group 2B. Ration : oaten chaff + bonemeal. Water : 10 p.p.m.F.	2nd pair in wear; all incisors show horizontal chalky bands, erosion, chalky areas and slight chipping of cutting edges.	3rd pair in wear, markedly eroded and stained over whole surface; some deep bands of pitting towards the gum mar- gin; broken cutting edge.	Ist pair shed; 2nd pair show increased staining; 3rd pair as previously described; 4th pair erupting.	2nd pair as previously described; 3rd pair show increased damage to cutting edge; 4th pair erupted at right angles, less chalky than other incisors but eroded and stained.			
C	Group 4A. Ration : oaten chaff. Water : 5 p.p.m.F + calcium sulphate.	3rd pair erupting; all incisors show marked horizontal striations, erosion and some chip- ping of cutting edges.	3rd pair in wear, markedly eroded and stained with deep bands of pitting.	lst pair, right only has been shed; 2nd pair show chalky striations over the whole surface and bands of pitting emphasised; 3rd pair markedly eroded and pitted and the right one at right angles.	lst pair, both have now been shed; 2nd pair right only has been shed; 3rd pair, the left one is now badly worn; 4th pair at right angles, eroded and stained.			

<b>a</b> 1		Comments on Incisor Teeth.						
Sheep.	History from 3 to 27 months of age.	At transfer to grazing. Age 27 months.	After 12 months' grazing. Age 39 months.	After 24 months' grazing. Age 51 months.	At slaughter after 30 months' grazing. Age 57 months.			
D	Group 4B. Ration : oaten chaff. Water : 5–7 p.p.m.F + calcium sulphate.	2nd pair in wear; all incisors are elongated, paper white with some chalky areas; crowding has produced an irregular incisor arch.	3rd pair erupting.	1st pair have been shed; 2nd pair show bands of pitting emphasised by staining; 3rd pair deeply eroded with staining over the whole surface; 4th pair in wear, erupted at right angles, ercded and stained.	2nd pair as previously described; 3rd pair as previously described; 4th pair as previously described.			
E	Group 6A. Ration : oaten chaff + peanut meal + lime- stone. Water : 5 p.p.m.F.	2nd pair in wear; all incisors show marked horizontal striations; 1st pair show some deep pits near the cutting edge; 2nd pair more chalky.	3rd pair erupting.	Ist pair have been shed; 2nd pair show increased staining; 3rd pair splayed, markedly eroded and stained; 4th pair, left only erupted at right angles.	2nd pair, staining empha- sises band of pitting at base; 3rd pair, as previously described; 4th pair, left only erupted at right angles.			
F	Group 6B. Ration : oaten chaff + peanut meal + lime- stone. Water : 10 p.p.m.F.	3rd pair erupting; 2nd pair chalky and heavily striated; 1st pair show marked surface erosion.	3rd pair in wear, markedly eroded and striated with chalky bands; 4th pair erupted at right angles, heavily eroded and stained.	Ist pair have been shed; 2nd pair have been shed; 3rd pair, right only has been shed, left only has been heavily eroded with bands of pitting; 4th pair as previously described.	3rd pair, left only as previously described, but shows a broken cutting edge; 4th pair chalky, eroded with some deep pitting.			

# Table 1.—continued. COMMENTS ON INCISOR TEETH—continued.

113

## Table 2.

### FLUORINE CONTENT OF MUSCLE, KIDNEY, FAT-FREE BONES AND TEETH.

(p.p.m.F on dry-matter basis).

Tissue.		Group 2A.*		Group 2B.†		Group 4A.*		Group 4B.‡		Group 6A.*		Group 6B.†	
		Group Average at 27 months.	Sheep A at 57 months.	Group Average at 27 months.	Sheep B at 57 months.	Group Average at 27 months.	Sheep C at 57 months.	Group Average at 27 months.	Sheep D at 57 months.	Group Average at 27 months.	Sheep E at 57 months.	Group Average at 27 months.	Sheep F at 57 months.
Gastrocnemius	 	2.0	1.5	1.0	1.5	3.0	1.5	2.0	2.5	3.0	1.0	1.6	3.0
Kidney	• • •	8.8	20.0	14.6	20.0	$9 \cdot 2$	28.0	16-8	52.0	26.2	16.0	43-4	35.0
Femur		1,300	1,320	2,600	1,800	1,600	840	1,600	800	1,030	1,080	1,750	1,240
Tibia	• •	850	1,040	1,700	1,360	1,275	880	850	880	550	1,040	1,450	1,200
Metatarsus		725	1,040	1,600	1,520	1,100	880	825	800	525	800	1,125	1,040
1st Incisors			Shed		Shed								
2nd Incisors		840	960	1,600	2,560	1,600	1,000	1,170	960	740	900		Shed
3rd Incisors		1,520	800	1,540	1,600	1,540	1,370	1,540	1,000	810	1,200	1,760	1,500
4th Incisors			470		550		725		600		600		1,270
3rd Premolar		960	1,360	1,520	2,400	1,300	1,440	1,240	1,040	690	1,120	1,460	1,526
2nd Molar	• •	780	880	960	1,520	920	1,200	920	1,120	400	1,120	1,160	1,120
3rd Molar		1,120	1,280	1,580	2,080	1,210	1,360	1,230	1,040	760	1,200	1,130	1,440
Mandible	• •	1,960	960	2,000	1,200	2,480	800	1,800	650	1,200	960	2,480	1,200
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\* On 5 p.p.m.F in drinking water from 3 months to 27 months of age.

† On 10 p.p.m.F in drinking water from 3 months to 27 months of age.

<sup>+</sup> On 5-7 p.p.m.F in drinking water from 3 months to 27 months of age.

J. M. HARVEY.

It was stated in discussing the earlier studies (Harvey, 1952) that the bone rarification noted after 24 months' exposure to fluorine may not have been entirely due to the fluorided water, and that other factors which may have contributed were the diet of chaffed or milled food and the confinement in pens. Further support to these conclusions is given by recent studies (unpublished data) on grazing sheep, in which bone rarification was not discernible at 33 months of age in a group continuously exposed to 10 p.p.m.F in the drinking water from the age of 3 months. From the present investigation it is apparent that the bone defects in these sheep are not permanent and have been largely overcome during the 30 months in which they had access to grazing or simulated grazing plus fluorine-free water. This is not true of the damage sustained by the incisor teeth. The dental lesions are permanent and the effects of erosion and wear are enhanced with age even though fluorine was excluded from the drinking water.

The fluorine concentration in the bones examined is in agreement with the X-ray studies. Comparison with fluorine levels found at autopsy after 24 months' exposure to fluorided water shows a marked reduction in the fluorine content, particularly in the mandible. This is in keeping with the marked increase in dense bone deposition during the 30 months when the animals were not exposed to fluorine.

The fluorine concentration in incisor teeth compares with levels recorded in similar sheep immediately after the exposure period. There has been a marked reduction in the fluorine content of the 4th pair of incisors when compared with that of the 3rd pair. This is particularly noticeable when the 4th pair did not erupt during the 12 months succeeding the exposure period. This is in agreement with the less marked lesions of fluorosis noted in this pair of incisors.

The fluorine levels in molar and premolar teeth tend to be higher than levels recorded immediately after the exposure period, when the eruption of the 3rd molars was in progress. This could be accounted for by wear on the exposed surface of the crown. The concentration of fluorine is much greater in dentine than in enamel, so any loss of enamel through wear on the masticatory surface of the tooth would result in an increase in the fluorine concentration in teeth of fluorotic sheep.

Consideration of the findings recorded in this study in relation to field practice stresses the importance of protecting sheep from fluorine during the susceptible age period when permanent teeth are being laid down. In marked distinction to bones, the effects of fluorosis on the incisor teeth are permanent. In fluorotic sheep incisor teeth erupting some 12 months after protection from fluorided water still show the pronounced lesions as well as positional defects.

### REFERENCE.

HARVEY, J. M. 1952. Chronic endemic fluorosis of Merino sheep in Queensland. Qld. J. Agric. Science. 9:47-141.





















 Incisors of Sheep A at (a) 27, (b) 51 and
 Incisors of Sheep B at (a) 27, (b) 51 and

 (c) 57 Months of Age.
 (c) 57 Months of Age.

## FURTHER STUDIES OF FLUOROSIS IN SHEEP.







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Fig. 3. Incisors of Sheep C at (a) 27, (b) 51 and (c) 57 Months of Age.

Fig. 4. Incisors of Sheep D at (a) 27, (b) 51 and (c) 57 Months of Age.

117





















Fig. 5.

Incisors of Sheep F at (a) 27, (b) 51 and (c) 57 Months of Age.

### FURTHER STUDIES OF FLUOROSIS IN SHEEP.



Fig. 7. Femur and Tibia of Sheep A at 57 Months of Age.



Fig. 8. Mandible of Sheep A at 57 Months of Age.

119



Fig. 9. Femur and Tibia of Sheep B at 57 Months of Age.



Fig. 10. Mandible of Sheep B at 57 Months of Age.



Fig. 11. Femur and Tibia of Sheep C at 57 Months of Age.



Fig. 12. Mandible of Sheep C at 57 Months of Age.



Fig. 13. Femur and Tibia of Sheep D at 57 Months of age.



Fig. 14. Mandible of Sheep D at 57 Months of Age.



Fig. 15. Femur and Tibia of Sheep E at 57 Months of Age.



Fig. 16. Mandible of Sheep E at 57 Months of Age.



Fig. 17. Femur and Tibia of Sheep F at 57 Months of Age.



Fig. 18. Mandible of Sheep F at 57 Months of Age.