Monitoring wool fibre diameter and length changes using gel emulsion autoradiography

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ABSTRACT

A technique for radio-labelling wool with using ^{35}S(cysteine) was modified by using a gel emulsion photographic film. The use of a gel photographic emulsion rather than sheet x-ray film reduces labour input and produces clearer autoradiographs. The autoradiographs produced are ideal for fibre diameter measurements at specific points in time, and for fibre length measurements between specific times. The adequacy of labelling using an intradermal injection was also studied. Provided that the area delineated for injection was not too large, adequate labelling of fibres was achieved. The use of a specific intradermal injection, rather than a systemic intravenous injection greatly reduces the cost of the procedure.

Keywords: Autoradiography; fibre diameter; Merino sheep.

INTRODUCTION

It is often necessary in studies of wool growth to relate changes in fibre diameter and fibre length growth to specific points in time such as a change in nutrition or physiological state, or the occurrence of a stress event. Downes *et al.* (1967) described an autoradiography technique which can be used to measure both wool fibre diameter at specific times and the length of fibres grown over a period of time. Their technique involved the intravenous injection of ^{35}S (cystine) followed by exposure of the wool fibres to an x-ray film to determine the points on the fibre corresponding to the time of injection. ^{35}S (cystine) was chosen as the isotope for injection as it was known to be incorporated rapidly into the growing wool fibre (Bern *et al.* 1955; Ryder 1956; Downes *et al.* 1962). Their method, while it has been used extensively, has several shortcomings, namely cost and labour intensity. The use of an intravenous injection rather than an intradermal injection requires a much greater amount of label to be injected and therefore greatly increases the cost of the procedure, in many cases making it prohibitive. The use of a sheet of x-ray film requires the film to be cut and superimposed back over the fibres after processing, making the procedure labour intensive.

Gel photographic emulsion has been used in recent studies (P. Hynd and S.Baker, personal communication) which removes the cutting and superimposing stages of the procedure. However, results have been variable, and in some cases no labelling has been detected. This paper outlines a procedure which has yielded consistently good results using a gel photographic emulsion.

MATERIALS AND METHODS

The wool samples for this study were derived from 20 Merino wethers, ten from each of NSW Agriculture's fleece plus (Fl+) and fleece minus (Fl-) selection flocks, which were part of a study examining the effect of rapid changes in the crude protein intake on fibre strength and diameter.

Wool clipping and isotope injection : Sufficient volume of L-[^{35}S]-cysteine solution (Amersham Aust.) was diluted with sterile saline (0.9%NaCl w/v) to supply 0.5μCi per 0.2ml injection at the estimated time of exposure. In practice this was routinely made up in 20ml batches in sterile injection bottles.

All sheep were clipped (Oster No. 10 blades) on the left hand shoulder region prior to the start of the experiment. This area was then clipped again using Oster No. 40 blades to leave a small "island" of wool about 2cm in diameter in the middle of the clipped area.

Each animal was injected with 0.2ml of the diluted radioisotope a total of 21 times throughout the experiment. A 1.0ml syringe fitted with a 25 gauge needle was used for the injection. Each injection was made intradermally into the middle of the area delineated by clipping. The intradermal injection was achieved by placing the needle at an angle of approximately 15° to the skin surface and inserting it until the bevel of the needle could no longer be seen.

Preparation of fibres for mounting : Three weeks after the experiment the 2cm diameter area was clipped from each animal. Each sample was washed in three changes of hexane before drying overnight. Each sample was then placed in contact with x-ray sensitive film (Hyperfilm, Amersham Aust.) for 48 hours, after which the film was developed for four minutes in undiluted Kodak D-19 developer. The film was then placed in a stop bath (3% acetic acid) for twenty seconds before fixation in Ilford Hypam fixer (diluted 1:4) containing Ilford Hypam hardener (diluted 1:40). Films were washed in running water for 30 minutes before drying. Once dry the film was superimposed over its' respective wool sample to identify the most intensely labelled areas, which were then removed for fibre sampling.

Fibre Mounting: Fibres were plucked from the selected samples and mounted on microscope slides which had been coated with a 10% gelatine solution containing 0.05% chrome alum. This was achieved by moistening the dried gelatine with a fine paint brush which had been dipped in warm distilled water. A fibre was then immediately plucked and mounted,

using the moistened paint brush to brush along the length of the fibre to ensure adhesion. Up to six fibres per slide could be mounted in this manner. After drying overnight, the slides were re-dipped in the warm gelatine solution to prevent fibre "lifting". At least sixty fibres from each sheep were mounted.

Exposure of slides: Slides were dipped in Ilford L4 Nuclear Emulsion diluted in distilled water and glycerol (140µl glycerol in 6.85ml distilled water made up to 14ml with the emulsion) and melted in a 43°C water bath under dark room conditions. Each slide was then placed on an ice-chilled metal tray for 10 minutes before drying for at least 2 hours in the dark at room temperature. The slides were placed in slide trays in a light-proof box containing silica gel. The box was wrapped in aluminium foil and black plastic bags to ensure the slides were not exposed to light, before storage in an isotope-free refrigerator at 4°C for as long as necessary for optimum exposure. Test slides were placed in a separate box for routine developing at weekly intervals to determine optimum exposure time.

Processing the slides: Boxes containing the slides were removed from the refrigerator and allowed to equilibrate to room temperature before processing. A blank control slide was processed before the experimental slides to check for "wrinkling" in the emulsion. If "wrinkling" occurred, the slides were left to equilibrate to room temperature for longer. Each slide was developed for two minutes in undiluted Ilford Phenisol developer, then rinsed in distilled water for ten seconds before fixing for two minutes in Ilford Hypam fixer (diluted 1:4) containing Ilford Hypam hardener (diluted 1:40). Slides were then rinsed through several changes of distilled water over ten minutes and allowed to dry. In practice the slides were placed in slide racks and processed in 2L beakers of the solutions. Slides were mounted using DPX mountant after rinsing in xylene.

RESULTS

Originally it was thought that the area of injection should be approximately 2cm in diameter. However, after the staples were exposed to the film, many of the staples within the injection area appeared to be unlabelled or only weakly labelled. Consequently, in a later experiment, the area of injection was reduced to approximately 1cm in diameter, resulting in more consistent and thorough labelling of the wool staples in the area (M. Friend, unpublished data).

The injection of sufficient ^{35}S(cysteine) to supply 0.5µCi of radiation per 0.2ml injection at the estimated time of exposure resulted in variable labelling. While some fibres and sites were strongly labelled, others were not. In addition, the time required for exposure was excessive (up to six months was required). In the subsequent experiment, the injection of sufficient ^{35}S(cysteine) to supply 0.75µCi of radiation per 0.2ml injection at the estimated time of exposure yielded far more consistent results after a few weeks exposure.

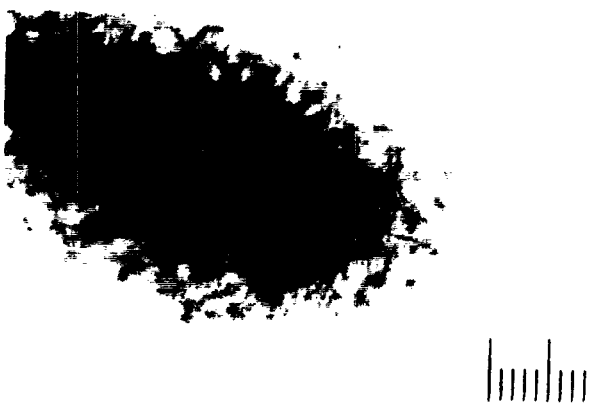
Diluted Ilford L4 emulsion yielded variable autoradiographs, and many of the images produced were not as intense as desired. The use of undiluted Amersham LM-1 emulsion yielded superior images.

Both emulsion manufacturers recommended developing slides in either Kodak D-19 or Ilford Phenisol diluted to the manufacturer's instructions. When slides were developed in either diluted or undiluted Kodak D-19 the results were poor. Silver grains could only be faintly seen in some areas. In addition, many slides had brown discolourations present. Even when the developer was made fresh on the day of use the same effect occurred. Other workers have reported similar results (C. Roberts, personal communication). After varying the time, temperature and agitation pattern of slides in the developer, results were no better. In contrast, the results obtained after developing slides in either diluted or undiluted Ilford Phenisol were far superior, regardless of whether the developer was freshly prepared on the day of use or not. However, severe "wrinkling" occurred when slides were developed in diluted Ilford Phenisol, an effect not observed when slides were developed in undiluted Phenisol.

The autoradiographs obtained by the gel emulsion method were extremely clear. In most cases the cuticle of the wool fibre could be easily seen (Plate 1). The autoradiographs produced when slides were processed using sheet film showed considerable scattering of silver grains making it generally harder to see the cuticle of the wool fibre (Plate 2).

PLATE 1: Autoradiograph of wool fibre obtained using the gel emulsion technique. The fibre and the emulsion layer are both in focus, and minimal scatter of silver grains occurs. The smaller crystal size also makes the image clearer (Scale: I unit =20 micrometres).

PLATE 2: Autoradiograph produced by exposing the fibres to a sheet of x-ray film which is then cut and mounted on the slide after processing. The exposure time is similar to the slide in Plate 1. The fibre and film cannot be viewed in focus together due to the thickness of the film. Note also the greater scattering of silver grains, and the greater crystal size (Scale: I unit =20 micrometres).



DISCUSSION

Even though many problems were initially encountered in using a gel photographic emulsion, after modification (including personal communications from P. Hynd and S. Baker), the method now produces consistently good autoradiographs, and the method is now routinely used in our laboratory.

The reduction in the area of the injection site from 2cm to 1cm in diameter is in keeping with the procedure for autoradiography using intradermal injections described by Orwin and Woods (1988). However it conflicts with the results of Downes *et al.* (1964) who observed that an intradermal injection of 0.5ml labelled an area of approximately 4cm in diameter. As these authors observed that the area of labelling was approximately proportional to the volume injected, an injection of 0.2ml would be expected to label an area of approximately 2cm in diameter. The increase in the amount of ^{35}S(cysteine) from 0.5μCi to 0.75μCi (both on the basis of an injection of 0.2ml and designed to yield these amounts of radiation at the time of exposure) is higher than doses used by other workers (P. Hynd and S. Baker, personal communications), and greater than the range suggested by Downes *et al.* (1967), but appears to be necessary to produce useable autoradiographs consistently.

It is not possible to say conclusively from our studies whether it is best to use the Ilford or Amersham gel emulsions, or whether the emulsions can be used effectively in diluted form. Both manufacturers suggest that their products can be used in diluted form, however our results are inconclusive. As we changed the area of injection, the amount of isotope injected and the type of emulsion used (as well as using it undiluted) from the first to second experiments it is not possible to identify the cause of the inferior results of our first experiment. However, the much greater exposure time required indicates that all four factors may have contributed. Amersham LM-1 is now routinely used as it is cheaper than Ilford L4 and can be obtained ex-stock, but it could be worthwhile to compare both emulsions in both diluted and undiluted form under the same conditions.

One of the major advantages of this technique relative to the technique described by Downes *et al.* (1967) is reduced labour input. Cutting and superimposing a sheet of x-ray film is very labour intensive as the film needs to be mounted on the microscope slide and then checked and repositioned daily until he mountant has set (S. Munro, personal communication). Nelson and Woods (1992) report a method using adhesive tape which reduces the labour input, however the technique they describe is still relatively labour intensive. The use of a gel photographic emulsion removes this step. Furthermore, it can be difficult to accurately superimpose the film over the fibres in the exact position it was while exposing. The use of a gel photographic emulsion removes the possibility of operator error on this step. As can be seen from Plates 1 and 2, the autoradiographs produced by using a gel photographic emulsion are much clearer than those produced using sheet film. This makes fibre measurements easier and therefore reduces the risk of operator error.

In addition, the use of a specific intradermal injection rather than a systemic intravenous injection greatly reduces the cost and potential hazard of the procedure as much less isotope is used. Downes *et al.* (1967) suggest an intravenous dose of 10μCi is sufficient for clear autoradiographs, although the dose needs to be increased for longer experiments as the half-life of ^{35}S is only 87.1 days. In recent experiments however, intravenous doses designed to yield 40μCi at the time of exposure have been required to produce useable autoradiographs after six weeks exposure (D. Tunks, personal communication). As the amount of ^{35}S injected in this technique is of the order of 0.75μCi at the time of exposure, both the cost and potential hazard of the procedure is greatly reduced. The need for disposal as radioactive waste of faeces and urine from injected animals is also not required when a specific intradermal injection is utilised. The claim by Downes *et al.* (1967) that in using intravenous doses over longer time periods increases the risk of missing doses has not been supported by other workers (Woods and Orwin 1988; Nelson and Woods 1992). In addition, the claim by Downes and Lyne (1961) that too many intravenous doses may damage the skin and alter wool growth rate has not been supported. P. Hynd (personal communication) intradermally injected sheep with ^{35}S (cysteine) at various intervals and volumes over an extended period of time. While visual skin damage was apparent, no change in wool growth rate was observed due to either the volume of isotope injected nor the interval between injections.

CONCLUSION

The use of gel emulsion autoradiography, in combination with an intradermal injection of the isotope, is a relatively quick and inexpensive way to monitor wool fibre diameter and length growth rate changes when experimental animals are subjected to changes in experimental conditions at specific times. Information generated from such studies will be valuable in developing management strategies to maximise wool growth and quality.

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