

THE EXPERIMENTAL PRODUCTION OF EPITHELIUM-LINED CYSTS*

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In a previous communication (Molyneux 1959) the bacterial degradation of wool fibres *in vitro* was described. The causative organism, an aerobic mesophilic spore-forming rod, was isolated from the contents of epithelium-lined cysts produced experimentally in sheep. This paper describes the production of these cysts by a technique which was developed in order to study the mechanism of cyst growth.

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The implantation technique involves the splitting of skin between the reticular and hypodermal layers of the dermis while cyst formation is ensured by maintaining the blood supply to the implant. The epithelium-lined cysts produced by this technique satisfy the following requirements:

- (1) Success of cyst formation.
- (2) Controlled initial size of the cyst.
- (3) Uniformity of cyst wall structure.
- (4) Maximum fluidity of cyst contents.
- (5) Maximum survival time of an intact epithelial lining.

Preliminary studies indicated that the above requirements for an experimental cyst were obligatory, if the mechanisms of cyst growth were to be adequately studied. Davis and Traut (1926) and Butcher (1946) formed epithelium-lined sacs by implanting full thickness skin in dogs and rats respectively. In both these studies, although cyst formation occurred, the cyst wall was not uniform in structure, the epithelium and cyst wall always being thicker over the area of the original graft. Because it was intended to make biopsies of the cyst wall at intervals, in order to study the relation between the increase in cyst size and histological changes observed in the epidermis and its derivatives, uniformity in cyst wall structure was essential. This uniformity has been achieved in the developed technique as shown in Plate 1, Figure 1. The cyst wall consists of full thickness skin and panniculus carnosus muscle, except at the area of closure (*A*) from which muscle, hair follicles, and sebaceous glands are absent.

The technique was developed in rabbits but was used in sheep (an animal provided with sweat glands) in order to increase the fluid content of the cysts so that intracystic pressures could be recorded.

Experimental

The technique for the production of epithelium-lined cysts was developed in albino rabbits of approximately 1.5 kg weight. The animals were anaesthetized with "Nembutal" given parenterally into the lateral ear vein. The mid-side skin of the animal was prepared by close clipping, defatting with ether and alcohol, washing in soap and water, and then applying "Cetavlon".

Circular areas 4 cm in diameter were outlined with gentian violet on skin overlying the lateral aspect of the thorax. An initial incision was made through the full thickness of the skin (extending from *A* to *B*, Fig. 1(*a*)). A curved incision *BCD* was then made through the epidermis and into the dermis to a level where, as described by Medawar (1944), the skin splits from its substratum. Medawar (1944), in describing the structure of rabbit skin, says: "The principal part of the dermis which underlies it [the epidermis], 0.40–2.00 mm in thickness, is composed of stout collagen fibres in the typical three-dimensional packing of compact connective tissue [reticular layer]. It grades superficially into the papillary layer, where the fibres are smaller and in more open packing; and below, rather sharply, into a layer where the fibres are orientated two-dimensionally in the plane of the integument [hypodermis]. At this deeper level, therefore, the skin 'splits' naturally from its substratum. The

principal arteries, veins, lymphatics and nerves of the integument travel in the fascial [hypodermal] layer, and they likewise run in the plane of the integument." Thus the incision BCD into the dermis was extended to a level between the reticular and fascial layers as shown by S in Plate 1, Figure 2. The principal vessels and nerves are contained in the deeper fascial layer which overlies the panniculus carnosus muscle. The incision was then extended from D to F through the full thickness of the skin, as for the incision from A to B .

Commencing at C the dermis was then split in the direction GH in the plane between the reticular and fascial layers. The splitting was continued until it covered the area indicated by the broken lines BG and DH shown in Figure 1(b). This distance should be sufficient to enable the eventual implant $BCDE$ to be drawn away from the proximity of the original suture line $ABDF$. The dissection frees from

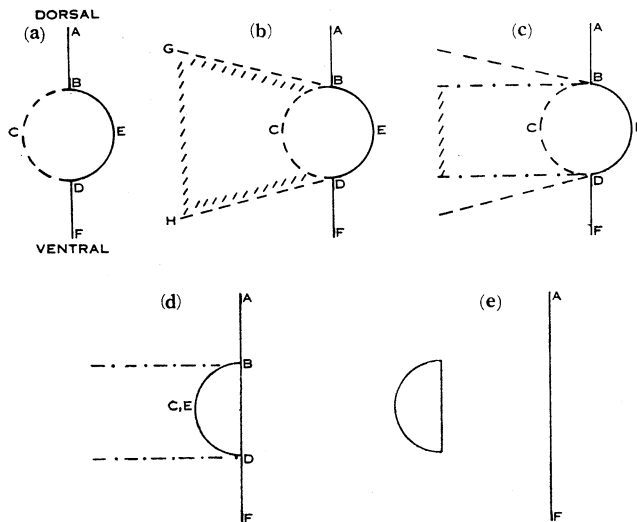


Fig. 1.—Diagram illustrating the method of preparing and placing the implants (see text for further explanation).

the fascial layer the epidermis and superficial dermis, leaving them attached to the fascial layer and blood supply along a perimeter indicated by the shaded area. An incision along the curve BED was then made through the full thickness of the skin. The skin to be implanted, $BCDE$, originally marked in gentian violet, can then be freed by blunt dissection from the loose connective tissue overlying the panniculus carnosus. This leaves it still attached to a vascular fascial pedicle, $BCDHG$, actually that part of the hypodermis lying between the superficial and deep planes of division shown in Plate 1, Figure 2. This skin can now be lifted free from the body wall. To free the implant further, making it freely moveable, the fascial layer was divided* as indicated in Figure 1(c), resulting in a narrowing of the base of the fascial pedicle.

* If this division is attempted earlier, difficulty will be experienced in stabilizing the implant during dissection.

The skin was then folded along the line *BD*, the free edges of the two semicircles *BED* and *BCD* being carefully apposed and sutured with fine non-chromic catgut. A closed semicircular sac lined by surface epithelium was thus formed (Fig. 1(*d*)). The implant can be transplanted either to a position between the reticular and fascial layers (area *BCDHG* in Fig. 1(*b*)) or beneath the fascial layer above the panniculus carnosus (by splitting this area in the deeper plane). In either of these positions it can be secured by a suture. The implant was placed laterally to the original suture line *AF* in order to minimize the tracking of infection from the surface to the cyst cavity.

To close the gap left by removal of the implant the surrounding tissues were freed by blunt dissection, the wound edges trimmed then closed in layers. The final result is indicated in Figure 1(*e*). This technique was then applied to sheep and seven implants were made in Merino and Corriedale sheep.

Examination of Cysts

The area of skin covering and surrounding the cyst was closely clipped as in Plate 2, Figure 1, and the cyst size measured with calipers. At implantation the cysts were semicircular but tended to become spherical during growth. The measurements, because they included the thickness of the surface skin, gave an indication of growth rather than an accurate record of size.

Biopsy was performed by the following method: An incision was made at a position adjacent to the cyst to the level of the panniculus carnosus (depending on the original depth at which the cyst had been implanted). The cyst was exposed by dissection, and by freeing the surrounding tissue the undersurface of the cyst could be turned towards the surface. The cyst was held firmly in this position while a biopsy punch 0.5 cm in diameter, similar to that used by Carter and Clarke (1957), was used to take a sample of the cyst wall (Plate 3). A biopsy punch 1 cm in diameter was used to take a control sample of surface skin adjacent to the cyst. Intracystic wool samples and the aspirated fluid contents of each cyst were also collected and examined.

Results

Seven implants were made in Merino and Corriedale sheep. Early cyst formation resulted in each case. Because of the presence of sweat glands, which remain active and apparently excrete into the cyst, the success of the implant can be judged within 10 days by the distention of the experimental cyst. There may be a large amount of fluid in the cyst; in one instance 12 ml of a brown clear fluid were aspirated. At intervals samples of cyst content, fluid and wool, were taken and biopsies of the cyst wall were made.

An example of an experimental cyst removed 10 months after implantation is shown in Plate 2, Figure 2. At formation the implant consisted of a semicircular (approx. 4 by 2 cm) double thickness layer of skin. At removal the cyst measured 6.5 by 4 cm. Because the cyst wall was uniform in structure, sampling by biopsy was possible and because of the thickness of sheep skin the dissection and fabrication of cysts were technically easier than with rat or rabbit skin, allowing a more accurate apposition of the graft-cut surfaces.

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EXPLANATION OF PLATES 1-3

PLATE 1

- Fig. 1.—Experimental cyst from rabbit skin with attached blood supply as seen after 7 weeks. *A*, area of surgical closure. Hair follicles are absent.
- Fig. 2.—Longitudinal section showing the full thickness of rabbit skin indicating the planes of surgical division. *ep*, epidermis; *dm*, dermis (papillary and reticular layers); *hy*, hypodermis containing blood vessels; *pc*, panniculus carnosus muscle; *S*, superficial plane of division for area *BCDHG* (Fig. 1(b)); *D*, deep plane of division for area *BCDE* (Fig. 1(c)).

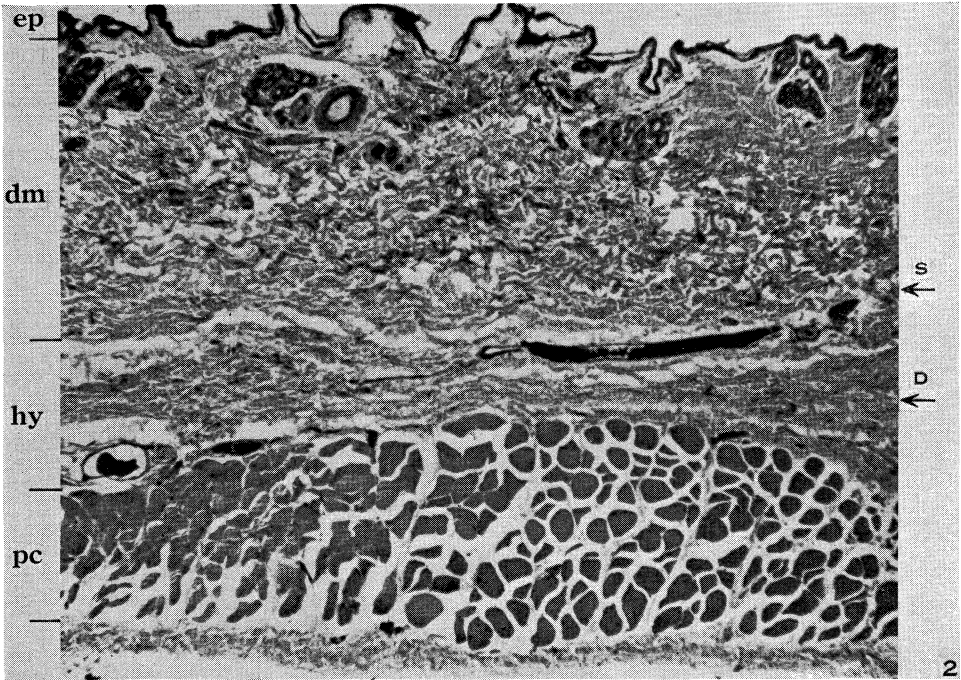
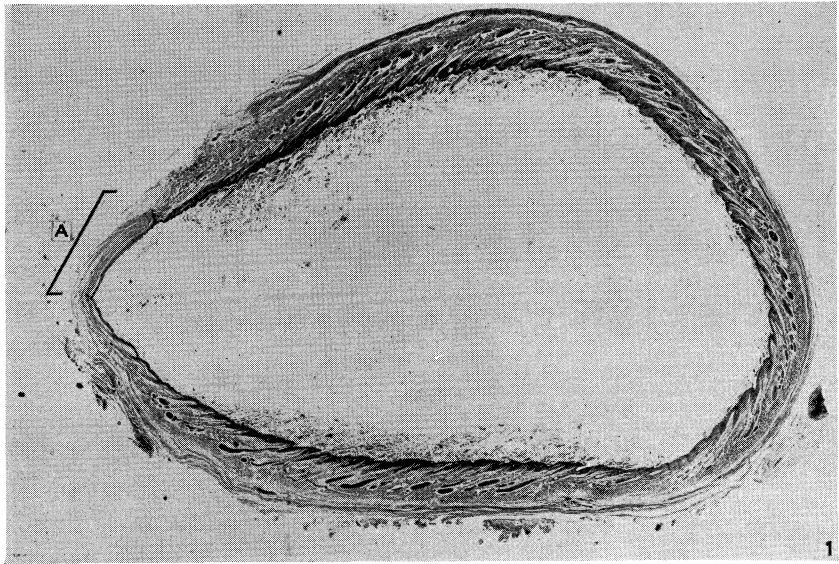
PLATE 2

- Fig. 1.—Mid-side of the trunk of a Merino sheep showing the position of three implanted experimental cysts.
- Fig. 2.—Experimental cyst from sheep skin with attached blood supply. The surface skin was left attached. This is the anterior cyst from animal shown in Plate 2, Figure 1.

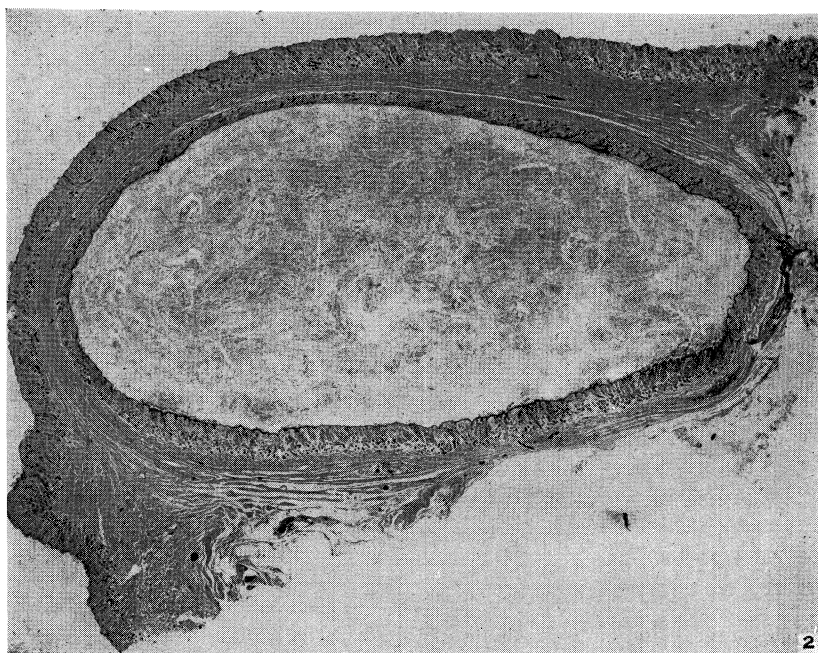
PLATE 3

- Fig. 1.—Experimental cyst from Merino sheep skin. Inverted cyst showing biopsy punch in position.
- Fig. 2.—Biopsy freed from the cyst wall but still attached to the intracystic wool.
- Fig. 3.—Biopsy completed and the tissue removed.

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