

viscid over a wide range of temperature, including 160° C. Silicone-lined tubes withstand dry heat and are relatively durable, not requiring to be recoated each time they are in use.

The toxicity of these substances and of fluids stored in silicone-lined tubes towards cells maintained *in vitro* was tested. The cells used included fibroblasts derived from the heart, nerve cells from the spinal cord, epithelial cells from the intestine, and cells from the spleen, all obtained from chick embryos. A total of seven hundred explants, including control cultures, was studied by the hanging drop method. Control and experimental cultures showed a close similarity in respect of rate of growth, contractility of muscular elements, cytological appearance and mitotic activity as determined by counts. These silicone compounds would appear, therefore, to be of value in tissue culture work.

As these materials are available in different grades, the sources of the varieties used in the tests are given below. Silicone rubber: DSR 210 (Dunlop Rubber Co., Ltd.) or 181 (Midland Silicones, Ltd.); silicone greases: stopcock and high vacuum (Dow Corning); silicone fluid: M 440 (Imperial Chemical Industries, Ltd.) for coating glass tubes to render them water-repellent. Preliminary tests on eleven other grades of silicone rubber with differing physical properties, such as elasticity, suggest that some of these may also prove to be non-toxic. The reaction of the tissues of the intact animal to some of these is now under investigation.

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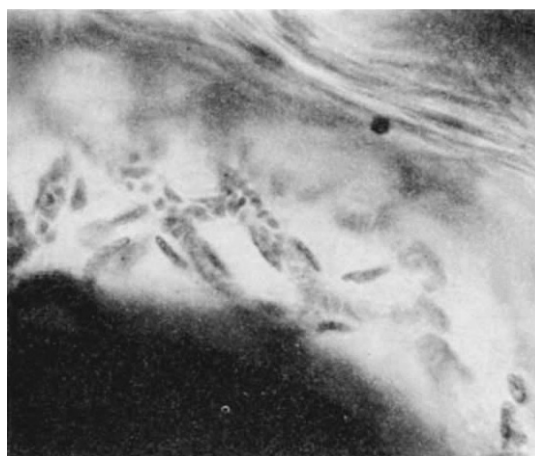
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Use of Cyanol in the Study of Blood-vessels in Sheep Skin

As one approach to the problem of the nourishment of the growing wool fibre in its follicle, a detailed study of the distribution of blood-vessels in the skin of sheep and the blood supply to the wool follicle has been commenced in these laboratories.

Because injection techniques are obviously unsuitable for adult animals, a staining method was sought. Various stains for haemoglobin have been used to visualize blood-vessels in histological sections (for example, that of Pickworth¹), but these have usually involved long and laborious procedures. The simple and rapid method of Dunn², which stains haemoglobin blue, nuclei red and cytoplasm pink, has not to my knowledge been used previously for this purpose. A slightly modified form of Dunn's method is being used, with good results, on thick sections, and it is thought that this might be of interest to other histologists. Whole-mount preparations have also been made by staining small pieces of skin in cyanol alone.

Dunn recommends fixation in 4 per cent formaldehyde solution buffered to pH 7.0. Although buffered fixative was not used here, the formalin was neutralized with sodium carbonate before the fixative (formol-saline) was prepared. It was found, however, that some specimens fixed in formol-saline which had not been neutralized in this way stained satisfactorily. According to the usual practice in this laboratory, the material was not taken below 70 per cent alcohol, after fixation in 5 per cent formol-saline.



Photomicrograph showing part of the capillary network around a wool follicle (dark area to the left). The light area around the capillaries is an artefact caused by the shrinkage, away from the follicle, of the connective tissue at the right. $\times 680$

Although Dunn states that the method is useful for wax sections, better staining was obtained with frozen sections, which show less shrinkage and are also quicker to prepare. Immediately after cutting at 100 microns on the freezing microtome, the sections are placed in 70 per cent alcohol, then stained in cyanol for up to five minutes and rinsed in 70 per cent alcohol (which removes cyanol from regions other than the red blood corpuscles). After this they are counter-stained with safranin for at least five minutes. The latter stain is differentiated in 70 per cent alcohol, and the sections are dehydrated in absolute alcohol, cleared in methyl benzoate and mounted in 'Distrene'.

These thick sections are excellent for demonstrating the distribution of blood-vessels and the general structure and arrangement of wool follicles and their accessory structures in the skin. Although the cyanol only shows blood-vessels when they are full of blood, this disadvantage is overcome because the safranin stains their walls, thus making them visible when empty.

Cellular detail of the vessel walls in the dermal connective tissue (the fibres of which are unstained) is very clear considering the thickness of the sections; different kinds of vessels (for example, arteries and veins) can be distinguished by the differences in structure of their walls. Cellular detail can also be seen in the glands; these finer details are, however, less easily seen in the thicker follicles, which are, of course, stained more densely.

Owing to their clarity, these preparations also show well melanin-containing cells. These pigment cells have been observed in the dermis of sheep skin and around blood-vessels as well as in the epidermis, around sebaceous glands, and in the outer root sheaths of follicles.

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¹ Pickworth, F. A., *J. Anat.*, **69**, 62 (1934).

² Dunn, R. C., *Arch. Path.*, **41**, 676 (1946).