A simple method for sealing surgical skin wounds in young or new-born mice

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Summary. To close skin incisions in albino mice, a solution of polymethyl methacrylate in chloroform is recommended to be smeared over the wound, a procedure which virtually eliminates the incidence of maternal cannibalism.

The need to find a simple method to close skin wounds postoperatively arose during our experiments with syngeneic albino new-born mice, 350 in number, which received s.c. thymus transplants. Under open ether anesthesia, transverse incisions, approximately 7–8 mm long, were made in the axillary region on both sides. The wounds needed to be closed well, particularly in view of the fact that the transplants had to be retained in place.

A small sample of the solution to be used to close the wound was prepared by dissolving 8 g of powdered transparent perspex (polymethyl methacrylate) in 50 ml of chloroform. The resulting solution is gelatinous in consistency. Since the skin of the new-born mice is quite freely movable, the approximation of the edges can easily be done by gently holding the animal's forelimb between the operator's index and middle fingers in order to fix the cranial lip of the wound and pushing the skin of the caudal lip from behind with the thumb towards the former. After thus bringing the edges of the wound together, a thin smear of the solution is painted over the wound. Any death due to anaesthesia was not taken into account.

The solution dries immediately due to evaporation of the chloroform. The dried mixture, which is smooth to touch and hard in consistency continues to hold the edges of the wound together. Even in those experiments where the muscle layer is to be stitched with the help of sutures[1], the skin can be safely closed with this solution. The dry smear is shed automatically once the wound is healed completely, usually between 3 and 5 days post-operatively.

If the mothers were fed enough just before carrying out the experiment on their litters, ample feed and water were kept in the cage, and the cage cleaned before returning the young ones, the operated animals could be safely transferred as soon as the smear dried up. This way, the loss due to cannibalism was found to be only 2%.

1 H.F. Helander and A. Bergh, Experientia *36*, 1295 (1980).

Immunoperoxidase localization of elastin in the human aorta

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Summary. Use of the peroxidase-antiperoxidase technique with preliminary trypsinization has allowed immunolocalization of elastin in human aorta. By this method it is possible to utilize formalin-fixed and paraffin-embedded tissue. The advantages in studying a strongly-autofluorescent material are discussed.

The study of elastic fibers by classical histochemical stains is hampered by considerable variation between different stains[1]. A specific immunological stain for elastin would be extremely useful, particularly for pathological tissues where changes in collagen and glycoprotein components often affect the staining reactions of elastic fibers[2]. Recently there have been reports of immunolocilization of elastin using ferritin-labeled antibodies in ultrastructural studies[3–5] but there have not been any reports of studies of human tissue at the light microscopic level using an immunoperoxidase technique. In previous work[6] we demonstrated the presence of elastin in frozen sections of unfixed human tissue by indirect immunofluorescence. However there are considerable limitations to the use of immunofluorescence in the study of elastic tissue. Such studies require the use of a microscope fitted with narrow wave band illumination, filters to cut autofluorescence to an acceptable level, and the use of frozen tissue has considerable practical limitations. We can now report immunoperoxidase staining of elastin in formalin-fixed paraffin-embedded tissue.

Methods. Antibodies to human foetal aortic elastin peptides were raised in sheep as described previously[6]. Specificity of the sera was checked by passive haemagglutination and radioimmunoassay[7]. No cross-reaction was detected with types I and III collagens and aortic glycoproteins.

Samples of adult human aorta from post-mortem examinations and surgical resections were fixed in 4% formaldehyde for 24 h, dehydrated, cleared in xylene and embedded in paraffin wax. 4 µm sections were mounted on glass slides and dried for 24 h at 37 °C.

Localization of antibodies to elastin was carried out using the peroxidase-antiperoxidase (PAP) method of Curran and Gregory[8]. Sheep PAP complex and rabbit anti-sheep IgG were purchased from Dynotech Ltd. U.K. Sections were dewaxed, rehydrated and endogenous peroxidase activity blocked by incubation with a solution of 0.5% H_2O_2 in methanol for 60 min at 20 °C. The sections were then digested with a solution of 0.1% trypsin (Gibco, U.K.) with 0.05% $CaCl_2$ in 0.05 M tris/0.4% NaCl at pH 7.8, for 45 min at 37 °C. The sections were rinsed thoroughly with tris-saline and incubated for 10 min at room temperature in a moist chamber with 25% normal rabbit serum in tris-saline to reduce nonspecific background staining. All subsequent incubations were carried out at room temperature with several washes with tris-saline between each incubation. The sections were incubated with anti-elastin serum or normal sheep serum at a dilution of 1:600 in tris-saline, washed and incubated with rabbit anti-sheep IgG at a dilution of 1:50. After further washing the sections were finally incubated with sheep PAP complex at a dilution of 1:40. These incubations were for 45 min each. The PAP stain was developed with a solution of 2 mg ethylcarbazole, 0.5 ml N,N-dimethyl-formamide in 9.5 ml acetate buffer pH 5 with addition of 2 drops H_2O_2. After 5 min the sections were rinsed, counterstained with haematoxylin and mounted in glycerin jelly.