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%.

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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.

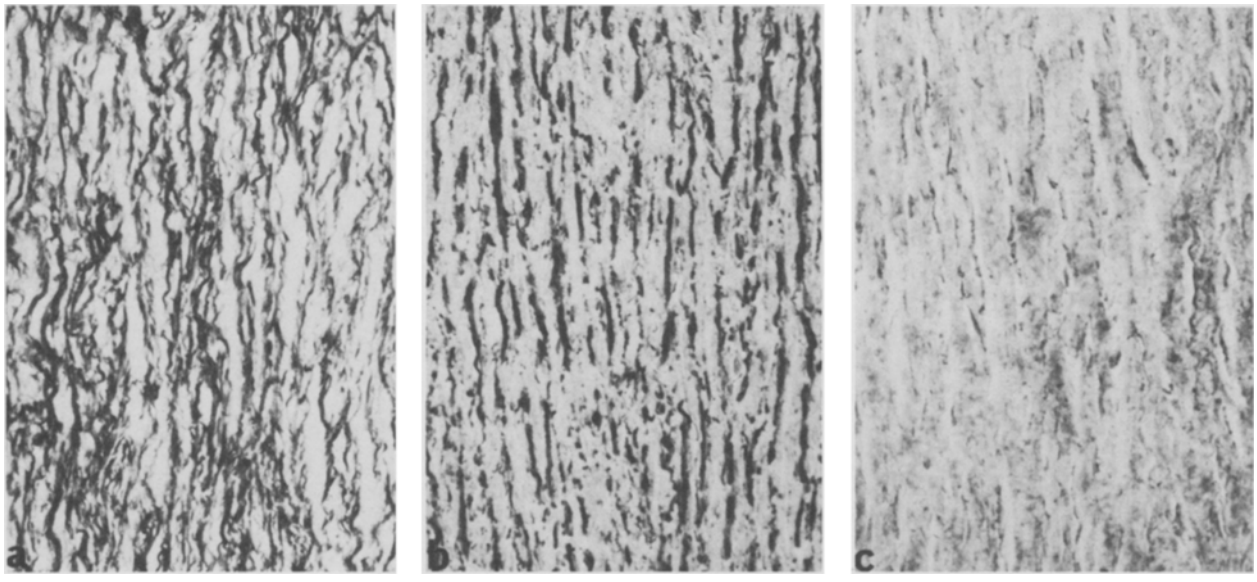

Figure 1. Medial coat of normal young adult male human aorta. *a* Vertically orientated medial elastic lamellae stained by Lawson's elastic van Gieson. *b* Elastic lamellae of the same aortic paraffin block after application of antielastin antiserum and PAP procedure. *c* Unstained but refractile elastic lamellae same aorta after application normal sheep serum and PAP procedure. × 175.

Blocking with α-elastin was achieved by preincubation for 24 h at 4 °C of 0.5 ml anti-elastin antiserum at a dilution 1:300 with 1 mg α-elastin peptides dissolved in 0.5 ml tris-saline. Similarly, blocking of the anti-elastin antiserum was attempted with a 1-mg solution of aortic glycoprotein extract dissolved in 0.5 ml tris-saline incubated for 24 h at 4 °C with the same volume of anti-elastin diluted 1:300.

Results and discussion. The anti-elastin antiserum showed immunostaining of the internal elastic lamina and deeper medial lamellae (fig. 1b) demonstrable by the conventional elastic-fibres stains (fig. 1a). The endothelium was unstained. Elastic fibers in the adventitia were also both stained by elastic fiber stains and by the anti-elastin antiserum. Normal sheep serum (fig. 1c) did not stain the internal elastic lamina, lamellae or adventitial elastic fibers.

The α-elastin peptides after incubation with the anti-serum achieved complete blocking of the immunostaining (fig. 2a and b). No inhibition of immunostaining was produced by the attempted absorbtion of the anti-elastin antiserum with the same concentration of aortic glycoproteins.

Elastin is an extremely poor antigen, being a highly cross-linked, hydrophobic and inert polymeric protein. Antisera to insoluble elastin are usually of a low titre, and although potent antibodies can be raised against tropoelastin (a soluble precursor to elastin) there are handicaps in the use of these for immunohistology of insoluble elastin. Firstly there is only partial cross-reaction between tropoelastin and insoluble elastin[9], and secondly, tropoelastin itself can only be isolated from the elastic tissue of animals treated with lathyrogens to inhibit cross-linking of the elastic polymer.

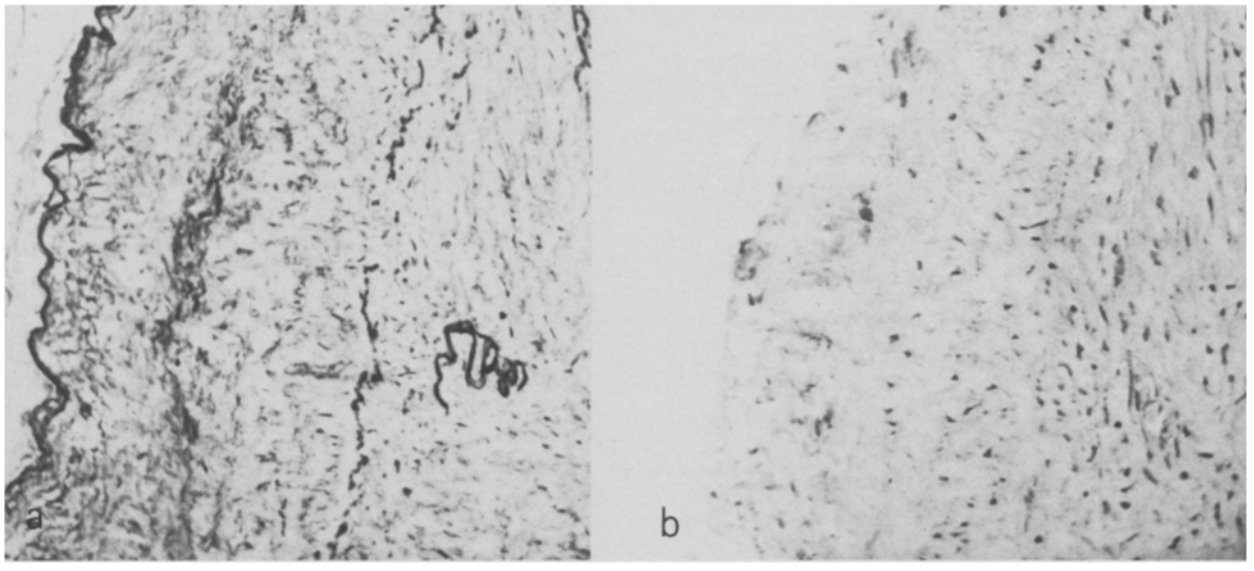

Figure 2. Human aorta from post-stenotic segment of aortic coarctation. Intact internal elastic lamina with fragmented and disorderly medial lamellae. *a* Antielastin antiserum and PAP stains elastic fibers of internal elastic lamina and lamellae. *b* Total suppression of staining after absorbtion of antiserum by α-elastin peptides. × 230.

Such a technique is not possible for human tissue and only limited cross-reactivity exists between elastins from different mammalian sources[7,10,11].

In contrast to the immunofluorescent method for demonstration of elastin antigens in human tissue, the peroxidase-antiperoxidase method has several positive advantages. It is possible to use formalin-fixed, paraffin-embedded tissue which is available stored in most histopathology laboratories. In this way the method obviates the need to prospectively collect and store in deep-frozen state tissues that are likely to be of interest. The enhanced autofluorescence of elastic fibers in such fixed tissue[12] precludes use of immunofluorescent method for its critical examination: the peroxidase-antiperoxidase method obviates this difficulty. Furthermore the peroxidase-antiperoxidase method allows examinations at intervals with relatively easy comparison between different tissue preparations.

We believe we have clearly demonstrated an immunological stain for human elastin in fixed tissue and believe this will be of great value in the study of diseases of human elastic tissue in a variety of different tissues[13].

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Quantitation of proliferative mucosal cells of the Mongolian gerbil on a weight basis[1]

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Summary. Quantitation of proliferative activity of the intestine of the gerbil shows a substantial decrease in proliferative activity as compared to the mouse. The use of dry vs wet weights proved to have no bearing on calculations of proliferative activity. The gerbil may prove a useful model for drug and radiation experimentation.

Use of the Mongolian gerbil, *Meriones unguiculatus,* as a research animal is expanding. Studies on the induction of tumors have been documented[2-5]; histocompatability factors[6] have been investigated, and others have done preliminary population kinetic studies[7-9]. The purpose of this study is to examine the gerbil's ileal cell population. Specifically we will report on quantitation of proliferative mucosal cells on a weight basis.

Materials and methods. Adult male gerbils (12–18 weeks old and weighing 52–66 g) were raised from 12 original mating pairs. The colony was derived from Tumblebrook Farms, West Brookfield Mass. and inbred for 2 years before animals were used for analysis.

Animals were injected i.p. with tritiated thymidine (^{3}HTdr 0.8 μCi/g b.wt, sp.act. 7.0 Ci/mM) 45 min before sacrifice. A small piece of ileum (3 cm) approximately 2.5 cm from the ileocecal junction was removed for analysis. The mucosa was scraped free from the muscle layer, weighed and used for scintillation counting using the Biological Material Oxidyzer (Oxymat Teledyne). The techniques of Hageman et al.[10] were used to determine the number of crypts and the number of proliferative cells per mg of ileum. A determination of cpm/mg was made for both dry and wet cellular weights. Quantitation used the following equations.

Number of crypts per mg intestine = A/B

Number of S cells per mg = A × C/B (where S represents the population of cells in the synthetic phase of the cell cycle)

$$\text{Number of proliferative cells per mg} = \frac{A \times C/B}{D}$$

Where

A = cpm/mg intestine

B = cpm/crypt

C = number of labeled cells/crypt

D = fraction of proliferative cells in $S(T_S/T_C)$ (where T_S represents the time of the S phase; T_C the time of the cell cycle)

Quantitation of proliferative activity of Mongolian gerbil mucosal cells on a weight basis

	cpm/crypt (B)[a]	cpm/mg (A)[b]	crypts/mg (A/B)	S cells/mg	$\left(\frac{A \times C/B}{D}\right)$ Proliferative cells/mg	$\left(\frac{A \times C}{B}\right)$
Dry wt		$1.0 \times 10^4 \pm 1.5 \times 10^3$	3.8×10^3	8.5×10^4	1.7×10^5	
Wet wt	2.67 ± 2.26	$1.6 \times 10^3 \pm 1.9 \times 10^2$	6.0×10^2	1.3×10^4	2.7×10^4	

Cpm/crypt + cpm/mg obtained from liquid scintillation counting. Other figures were derived from equations. [a]SE based on 15 animals. [b]SE based on 7 animals.