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A new radio-opaque injection technique for tissue preservation

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Summary—This study has investigated the action of two commonly available preservatives, chlorbutol and chlorocresol, on fresh cadaver tissue. If the arterial system of the subect is perfused first with either preservative then the progression of tissue necrosis can be delayed for up to 1 month. The preservative can be combined with the lead oxide-gelatin mixture described by Rees and Taylor without compromising the results of radiographic studies. Both chlorocresol and chlorbutol have the advantage over formalin of retaining the normal colour and texture of the tissues. A regimen is suggested which has all of the advantages of the mixture originally described by Salmon, but is much simpler and cheaper to produce. It has proved successful when used in whole cadavers but the visceral contents must be removed at an early stage.

For more than a decade there has been an intensified reappraisal of the blood supply of various tissues in order to define new donor sites for tissue transfer and to modify and improve conventional operations. In the past our investigations have concentrated on isolated regions of the body and the best results have been obtained by dissection of fresh cadavers, especially when combined with radiographic studies of a suitably injected substance.

Recently our investigations expanded to encompass the entire body (Taylor and Palmer, 1987). Because each study required a prolonged period of dissection, we needed to identify a preservative which could be combined with our radio-opaque mixture and which would retain the normal colour and texture of the tissues. Formaldehyde was unsuitable since it altered the tissue colour, it made the tissue planes rigid and the dissection of small vessels difficult.

In 1936 Salmon described a radio-opaque preservative which he used in fresh cadavers. It included lead-oxide Pb_3O_4 (minimum), the preservative phenol, and held the tissues in a good state for about a month. We had attempted to use this mixture in our earlier studies of isolated tissues but found it too tedious to manufacture, temperamental to use and expensive. Consequently we modified and simplified Salmon's mixture (Rees and Taylor, 1986). The litharge (PbO) form of lead oxide was substituted for Pb_3O_4 to which was added gelatin and water.

This simple mixture produced excellent results. However, a preservative had not been included since the duration of each of our regional and isolated tissue studies was too short to require its addition.

Therefore a preservative was sought for our total body studies which was inexpensive, could be substituted for the water in our modified version of Salmon's mixture, would retain the colour and texture of the tissues, would not alter the radio-opaque nature of the lead oxide or prevent the gelatin from solidifying the mixture to a rubbery consistency.

To this end chlorocresol and chlorbutol were investigated.

Materials and method

Chlorbutol and chlorocresol are disinfectants commonly used as medical preservatives (Martindale, 1982). Both are soluble in normal saline and water and it therefore seemed likely that they would be compatible with a mixture of gelatin and red lead.

Chlorbutol ($C_4H_7Cl_3O_{1/2}H_2O$) has molecular weight of 186.5 and exists in the form of colourless or white crystals at room temperature. It has a solubility in water of 1:130 and is more stable in acidic solutions. Although it is used as a sedative, it has antibacterial and antifungal properties when used in concentrations of 0.5%. At this concentration it is close to its saturation point and at low temperatures crystallization may occur.

Chlorocresol (C_7H_7ClO) has a molecular weight

Figure—Radiographs of various tissues injected with the lead oxide-gelatin mixture containing preservative. (A) gastrocnemius muscle. (B) segment of small bowel. (C) gall bladder and biliary ducts. (D) sciatic nerve. Note that the vessels interconnect in all of these tissues to form a continuous network as highlighted in our paper on the angiosomes of the body (Taylor & Palmer, 1987).

of 142.6 and is also in the form of colourless crystals at room temperature. It has a characteristic phenolic odour and is soluble in water to 1:260. Chlorocresol is much less toxic than phenol. It is a potent disinfectant and is more active in acidic than alkaline solutions.

Fresh cadavers were examined within 6 to 24 hours of demise. Because whole bodies were to be studied in our definitive work, it was important to know how soon the viscera would have to be removed as well as the preservative effect on the muscle, skin and other soft tissues. The investigations therefore were conducted on muscle, skin, liver and intestine after perfusion or immersion with chlorbutol 0.5% or chlorocresol 0.3% dissolved in water. The tissues were stored at 4°C and inspected daily. They were assessed for appearance, odour and texture. Radiographs were made of those tissues perfused with the lead oxide preservative mixture as well as histological studies to assess the extent of microvascular perfusion.

Musculocutaneous studies

The gastrocnemius musculocutaneous flap was selected as the model and 30 limbs were examined. In most subjects both legs were studied to provide a control or a comparison between preservatives.

In five cadavers the gastrocnemius muscle and overlying skin were removed from one limb as controls, stored and assessed as outlined above. In the remaining 25 studies the limb was perfused via the common iliac artery before removing the musculocutaneous samples. A volume equal to twice the predicted vascular volume of the leg was used. This was based on previous estimates by Rees and Taylor (1986) and was usually between 200 and 400 ml.

In 10 cases the limb was perfused with preservative alone, 5 with chlorbutol and 5 with chlorocresol. In another 11 cases the limb was perfused with a preservative and this was followed with the lead oxide mixture containing the same preservative. In 5 of these the preservative was chlorbutol and in 6 it was chlorocresol.

In the remaining four cases the limb was flushed first with saline. The chlorbutol lead oxide mixture was then introduced in two limbs and the chlorocresol counterpart in the remainder.

The red lead, gelatin and preservative mixture was heated to 50°C in a water bath in each case and injected in a pulsatile manner. One to two hours was allowed for the mixture to set. The specimens were removed, X-rayed, stored and analysed.

Liver and intestine studies

Sixteen specimens of intestine and eight sections of liver were analysed. In five cadavers, two segments of small bowel were removed and emptied of their contents.

One sample from each body was immersed in chlorocresol and the other in chlorbutol. In another cadaver two specimens were removed, ligated at each end to keep their contents *in situ* and stored, one in each preservative. In two cadavers, four segments of bowel similarly ligated at each end were injected via the artery in their vascular pedicles with the contrast mixture containing either chlorocresol or chlorbutol. Three of these samples had no further treatment before dry storage, but one was kept immersed in chlorocresol solution.

Eight liver sections were taken from four cadavers. One sample from each body was immersed and stored in chlorocresol and the other in chlorbutol.

Results

Tissue perfusion

Radiological assessment of the specimens injected with the lead oxide, gelatin and either preservative revealed satisfactory filling of small vessels. The results were comparable with those described previously by Rees and Taylor (1986) where a preservative was not included in the mixture. They are shown in the figure, together with specimens of other tissues taken from our subsequent whole body studies.

Histological analysis demonstrated filling of arterioles and in some cases capillaries. This was dependent on the gelatin content and hence the viscosity of the mixture. It was not influenced by the presence or absence of the preservative.

Tissue preservation

The preservative effect of the chlorocresol and chlorbutol on muscle and bowel are shown in Tables 1 and 2.

Muscle: The control muscle deteriorated in 3 weeks when kept at 4°C. To our surprise so did the muscle treated with saline preperfusion followed by either preservative in the lead oxide mixture. However, preperfusion with preservative delayed muscle decay for approximately 4 weeks. Chlorocresol was marginally better than chlorbutol.

It is of interest that this period was prolonged for a further week when the preservative only was used.

Table 1 Preservation of muscle and skin

Limb perfusion	*Number*	*Muscle and skin preservation time (days)*
No perfusion (Controls)	5	20
Saline then Chlorocresol-lead, gelatin mixture	2	18–22
Saline then Chlorbutol-lead, gelatin mixture	2	18 22
Chlorocresol then Chlorocresol-lead, gelatin mixture	5	26–30
Chlorbutol then Chlorbutol-lead, gelatin mixture	5	24–28
Chlorocresol only	5	35
Chlorbutol only	5	35

Table 2 Preservation of intestinal segments

Contents	*Preservative injection*	*Immersion*	*Number of samples*	*Intestine preservation (days)*
Removed	None	Chlorocresol	5	35
Removed	None	Chlorbutol	5	35
Present	None	Chlorocresol	1	up to 5
Present	None	Chlorbutol	1	up to 5
Present	Chlorocresol	None	2	up to 5
Present	Chlorbutol	None	1	up to 5
Present	Chlorocresol	Chlorocresol	1	up to 5

In one cadaver a septicaemia had been present prior to death. The muscle sample from this subject is not included in the tabulated results. Despite preperfusion with chlorocresol and subsequent perfusion with the preservative contrast mixture, it deteriorated rapidly within 14 days.

Intestine: This highlighted the potential pitfall for our future whole body studies. The intestine decomposed within 5 days, regardless of the preparation, when stored at 4°C with its contents *in situ*.

However if its contents were evacuated first it was sustained in either preservative for 35 days. This compared with the results obtained with the liver samples.

Discussion

Our results revealed that either chlorocresol or chlorbutol could be combined with lead oxide and gelatin without affecting the physical properties of the mixture, its ability to perfuse the small radicles of the arterial framework or the subsequent radiographic studies. However, they had no preservative effect unless they were injected first and when this was done they prolonged muscle survival for approximately 1 week. Notably the chlorbutol and the chlorocresol retained the normal colour and texture of all tissues thus facilitating dissection.

It is interesting that when the preservative-lead oxide mixture was used without prior perfusion with either chlorbutol or chlorocresol, the musculocutaneous sample deteriorated in the same time as the controls. The gelatin sets quickly and perhaps the preservative is trapped within the gelatin lattice before it has a chance to diffuse into the tissues and take effect. Undoubtedly preperfusion with preservative delayed tissue decay.

In our studies the lead oxide preservative mixture was introduced immediately after the initial perfusion with chlorbutol or chlorocresol. Therefore it may be wise to wait for a period before introducing the lead oxide mixture. This is substantiated by the fact that chlorbutol or chlorocresol alone produced the best results.

The gut is the weak link in the preservation of a whole cadaver as the intestine deteriorates within 5 days with its contents *in situ*. The results of immersion of the intestinal segments in either chlorbutol or chlorocresol with their contents *in situ* would suggest that there would be no advantage in filling the abdominal cavity with either preservative.

Based upon the information obtained from this pilot study we adopted the following regimen in six whole body fresh cadaver studies, the results of which are published elsewhere (Taylor and Palmer, 1987).

The body was first irrigated with 0.3% chlorocresol via one femoral artery. This was followed by lead oxide and gelatin dissolved in the same preservative. The subject was cooled to 4°C and the viscera were removed in each case within 5 days. Additional time was gained by removing the integument and storing it in a deep-freeze compartment while the underlying tissues were dissected. In this way we were able to complete the deep tissue dissection within one month and to examine the integument at leisure.

Conclusion

The preservative effects of chlorbutol and chlorocresol have been demonstrated on fresh cadaver tissue. They are compatible with our lead oxide

gelatin mixture but they execute their preservative effect only if injected first.

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