

Technical method

Use of liquid paraffin in the preservation of pathological specimens

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Since the introduction of colour preservation methods for the mounting of pathological specimens at the end of the nineteenth century, numerous modifications have been advocated, but there has been little real improvement on the original method of Kaiserling (1900). This is still by far the most satisfactory method, but the process is largely an art in which the selection of a particular recipe depends very much on individual preference and custom.

In this laboratory we have used a weaker formalin in the first fixative solution than the 20% formalin recommended by Kaiserling. We have also found that a higher concentration of sodium acetate and a stronger solution of glycerol in the third, final, preservative solution, in which the specimens are mounted, gives better results than the strengths he advised. The stronger glycerol solution provides the optimal density for use with the Perspex jars that are now invariably used for mounting specimens.

Useful as this method has proved, provided the material supplied is fresh and properly displayed, we have encountered a number of difficulties. One has been a tendency for the specimen to shrink, and another for large specimens of skin to become translucent. The greatest drawback, however, has been the tendency for pigments, such as haemoglobin, bilirubin, melanin, and lipochromes, to seep out of the specimen into the preservative solution, which becomes progressively discoloured. This necessitates a changing of the fluid, in some instances repeatedly within the span of a few months, over a period of many years. Not only is this a waste of preservative fluid, but even more serious is the gradual loss of the specimen's distinctive colour so that it becomes dull and nondescript. The organs especially susceptible to such loss of coloration are the liver, gallbladder, spleen, and kidneys, as well as tissues bearing pigmented tumours. To obviate this

loss of pigmentation we have investigated liquid paraffin as a preservative solution instead of glycerol and water.

Material and methods

The composition of the three solutions used is as follows: the first fixative solution consists of potassium nitrate 11.25 g, potassium acetate 21.25 g, formalin 100 ml, and water 1000 ml; the second colour restorative solution is 64 OP industrial spirit (approximately 95% ethanol); the third preservative solution is pure liquid paraffin.

The pathological specimen is placed in the fixative solution for a period ranging from 48 hours for thin material, such as a piece of intestine, to three weeks for an amputated limb. The usual period of fixation for pieces of solid organs is seven to ten days. The specimen is then placed in the colour restorative solution, where it is also washed free of fixative solution, until the original colour is restored as fully as possible. This usually takes a few hours. In any case the specimen should not be kept in the solution for longer than six hours, otherwise progressive shrinkage will take place. The specimen is then transferred into the preservative solution, in which it is finally mounted in a Perspex jar.

Results and discussion

This method has been used for one year, and the results have been very satisfactory. The optical density of liquid paraffin compares favourably with that of the glycerol solution used in the Kaiserling method. Furthermore, there has been little tendency for the preserving fluid to become discoloured by pigment seeping out from the specimen. Conversely, the specimen has retained its colour far better than in the glycerol solution, and its structure is more sharply delineated. All the major organs of the body have been preserved in liquid paraffin, and none has reacted adversely to the treatment. The best results have been noted in solid organs full of blood, such as the spleen, liver, and kidney, or those which are deeply pigmented, such as gallbladders, kidneys with clear-cell carcinomas, adrenals with cortical carcinomas or pheochromocytomas, skin with malignant melanomas, and deeply bile-stained livers. In one such liver, the seat of primary biliary cirrhosis, the first mount of preservative solution was rapidly discoloured, the bile pigment material

sinking to the bottom of the jar, but the second change of the solution has remained clear while the liver remains dark green in colour.

A considerable number of old specimens previously mounted in the Kaiserling sodium acetate glycerol preservative solution, which had undergone repeated discoloration after the fluid had been changed, have now been remounted in liquid paraffin. In the great majority there has been no further loss of pigment, but in one specimen of spinal column the blood pigment has continued to leak into the liquid paraffin. In a few a small amount of pigment has formed a thin sediment at the bottom of the jar.

It seems that the use of liquid paraffin as a final preservative solution in which pathological material is mounted will obviate much of the discoloration of the mounting fluid that so detracts from the appearance of pathological specimens in medical museums. Not only will the specimen look more lifelike, but there will also be a considerable reduction of

expense when less satisfactory Kaiserling preservative fluid, which has to be changed repeatedly, is replaced by the essentially inert, non-reactive liquid paraffin. It should be noted that 25 litres of liquid paraffin (British Drug Houses) cost £23.93 as opposed to £35.67 for a similar amount of glycerol (Fisons).

This method has been tried on fresh specimens for only one year, which is admittedly too short a time for final evaluation; many another promising technique has been proved wanting by the passage of time. However, we first tried liquid paraffin eight years ago on an enormous old spleen with chronic myeloid leukaemia, and the organ is still in a state of good preservation.

Reference

Kaiserling, C. (1900). Über Konservierung und Aufstellung pathologisch-anatomischer Präparate für Schau- und Lehrsammlungen. *Verhandlungen der Deutschen pathologischen Gesellschaft*, 2, 203-217.

Letters to the Editor

A comparison of viral transport media

Following the report of Chaniot *et al.* (1974) that storage survival of some respiratory viruses was increased when HEPES rather than bicarbonate buffer was used in media, we report an initial comparison of the survival of herpes simplex virus (HSV) at room temperature in viral transport media (VTM) comprising Hanks' balanced salt solution (HBSS) and 0.2% bovine albumin, buffered (pH 7.2) with HEPES and bicarbonate respectively. When bottles of each were inoculated with a field strain of HSV to a concentration of 63 TCD₅₀/ml, viable virus was detected for 29 days in the former and for 21 days in the latter. Increasing the bovine albumin concentration to 1% in the bicarbonate VTM resulted in virus detection for 17 days, and when 0.425% lactalbumin hydrolysate was also included, for 15 days. Comparisons of the survival of respiratory syncytial virus (320 TCD₅₀/ml) in HEPES VTM and one

with HBSS, 1% bovine albumin and 0.425% lactalbumin hydrolysate showed survival of viable virus for at least 13 days in the former and for only three days in the latter.

These initial findings suggest that (a) the use of HEPES buffer results in longer virus viability, and (b) neither increasing the bovine albumin content nor adding lactalbumin hydrolysate significantly prolongs survival of labile viruses in bicarbonate buffered VTM. The increased cost of using HEPES instead of bicarbonate buffer is 33p/litre.

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Reference

Chaniot, S. C. M., Holmes, M. J., Stott, E. J., and Tyrrell, D. A. J. (1974). An investigation of media for the long term storage of three respiratory viruses. *Archiv für die gesamte Virusforschung*, 44, 396-400.

Diagnosing thalassaemia trait from platelet count and England's discriminant function

We are very interested in Hegde's recent paper (Hegde *et al.*, 1977) reporting that a thalassaemia trait may be shown using England's discriminant function (DF): $DF = MCV - (Hb \times 5) - RBC - 304$ (1), calculated from indices obtained on a Coulter Model S, if the levels of Hb A₂ and Hb F are within normal limits. It appears, however, that this function is unable to distinguish between all cases of thalassaemia. England (England *et al.*, 1973; England and Fraser, 1973) found a negative value of the DF to be significant for iron deficiency; in Hegde's cases, the DF is positive in 7 of 13 confirmed a thalassaemia traits, in 19 of 57 suspected a thalassaemia traits, and in 3 of 20 β thalassaemia traits, that is, in about 30% of the heterozygous thalassaemias.

We reported (Seigneurin *et al.*, 1977) that the thrombocyte blood count used