of slides were used, one of each pair being processed with the usual diluent and the other in diluent containing 10% acetone. Acetone had no effect on the quality of staining.

Discussion

The results we obtained with the new equipment indicate that it can greatly reduce the time taken to process large numbers of slides, while retaining a high degree of quality. The equipment could be constructed for any number of slides, but is increasingly worthwhile when a dozen or more slides are processed. Readily available materials have been used, and the design has been aimed at simplicity of construction that is well within the capabilities of an average hospital carpentry department.

Other advantages are the rinsing of chromogens of dubious safety before touching the slides and the loss of fewer sections from the slides. We believe that the

usefulness of this equipment will find wide appeal among clinical pathologists and research workers.

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Microwave polymerisation of epoxy resin: rapid processing technique in ultrastructural pathology

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The problem of rapid processing in biological electron microscopy has often been addressed, particularly in relation to diagnostic pathology. Overnight processing schedules may now be regarded as normal, but although considerably shortened schedules are possible, our experience suggests that some loss of quality is evident when total processing times with standard epoxy resins are less than about three hours. Using conventional oven techniques, the polymerisation step, traditionally one of the longer elements in the process, needs to be at least half an hour. Recently there has been increasing interest in the use of microwave irradiation for tissue fixation,^{1,2} but although its use in dental practice³ and industry⁴ has been addressed, its possible application to polymerisation in electron microscopy seems to have been ignored. This paper shows that microwave polymerisation is readily practicable and that it may have distinct advantages over conventional procedures.

Material and methods

All tissue in this study was fixed in 4% phosphate

buffered glutaraldehyde. A standard pre-polymerisation schedule, taking four and a half hours, was used. Medium hardness EMIX resin and EMcap embedding capsules (EMscope Ltd) were used. Control polymerisation was achieved by heating overnight (16 hours) in a 70°C oven, while tests were carried out in a Sharp microwave oven (Model R4060) of 400 W output rating at 2450 MHz. All timings were undertaken manually as the intrinsic timer was too inaccurate for the short time intervals concerned.

The two primary tissue samples chosen for this study had been fixed for more than 24 hours. An additional fresh tissue sample was subjected to 60 seconds of microwave assisted fixation in glutaraldehyde, according to the technique of Leong *et al*² before being subjected to the same preparation and polymerisation schedules as the primary test samples. Conventional sectioning staining and photographic procedures were used throughout.

Results

The microwave field within the oven was found to be inhomogenous, with a particularly strong central zone. Such non-uniformity, although relatively unimportant where large volumes of liquid are being heated in the centre of the oven, is of major concern when using very small volume embedding capsules that can be placed anywhere within the oven. We have not yet made a detailed analysis of field strength and distribution, but we found that consistent and useful results were obtainable with capsules placed in a row at the front of the oven.

Uninterrupted exposure to the centre of the micro-

Fig 1 (a) Micrograph from conventionally polymerised block of tissue from secondary deposit of malignant mesothelioma. Adhesion specialisations, microvillous profiles and granular endoplasmic reticulum are prominent features. $\times 17\,000$. (b) Micrograph from microwave polymerised block of tissue from same tumour as (a). Same organelle profiles are equally clearly defined. $\times 17\,000$.

wave field for one minute or more produced almost explosive polymerisation with distortion of the embedding capsule through excessive heating; similar exposure at the front of the oven produced overcuring with brittle blocks. Cumulative short exposures with seven 15 second pulses and two 30 second pulses separated by 30 second cooling intervals gave very acceptable and consistent polymerisation. After polymerisation blocks were rapidly cooled in a refrigerator at 4°C. Fig 1 shows a comparison between a block of tissue subjected to this technique and a block of tissue polymerised by conventional means, but

derived from the same pathological specimen and otherwise treated identically. No noticeable differences in quality were evident, and the cutting qualities of the microwave polymerised blocks seemed to be in no way inferior to those of conventional specimens, although blocks more than 2 mm across displayed slightly uneven polymerisation, while marginally undercured blocks were hard in the centre but had tacky surfaces, particularly at the tip. Fig 2 shows the very acceptable quality obtained with a sample subjected to both microwave assisted fixation and microwave polymerisation.

Fig 2 Diffuse centrocytic/centroblastic lymphoma. Material subjected to microwave assisted, glutaraldehyde fixation and microwave polymerisation of epoxy resin embedded block. $\times 10\,000$.

Discussion

This study shows that microwave polymerisation is practicable. Heating and curing is extremely rapid, probably on account of hydroxyl groups present in the uncured resin, or formed during curing and through exothermic reactions that sustain heating after the microwave source has been switched off. Indeed, careful control is required to eliminate boiling effects.

Although its general usefulness remains to be established, this technique may be of value in two ways. Firstly, if uniform fields can be applied, short microwave pulses with variable time intervals are likely to permit more precise control of polymerisation than is possible with conventional ovens, thus leading to greater flexibility and consistency. Secondly, the ability to polymerise conventional blocks within minutes reduces the time for rapid processing schedules without incurring any penalty in quality of preparation.

Fig 2 shows that substantial time reduction is possible at each end of the processing cycle, although it is recognised that resin infiltration is likely to prove the time limiting step. While it may prove impractical to reduce total processing time below an hour or so, the studies of Boon *et al*⁵ suggest that, even here, microwaves may be of assistance. If processing times of one to two hours can be achieved without any reduction in quality the consequent flexibility in processing, particularly if automated, is likely to be a major advantage in the routine laboratory. The technique could also be applied to plastic embedding and sectioning of small samples in conventional histopathology and might prove useful in the preparation of urgent speci-

mens which arrive at an inconvenient time of day.

For consistent results on a larger scale, ovens with a more uniform field, such as may be provided by a rotating antenna—for example, may be desirable. Furthermore, electronic programming of the pattern of pulses and cooling intervals would be a considerable advantage. Ultimately, a custom built module for both microwave fixation and polymerisation may be the most appropriate solution and this is under active consideration.

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