

Technical methods

Histological fixation by microwave heating

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Heat can effect the partial denaturation of protein that is the basis of histological fixation. An objection to conventional forms of heating is that only small specimens can be used, as heat conduction is poor in biological materials.

Microwave heating overcomes the limits imposed by poor heat conduction. Microwave energy is part of the electro-magnetic spectrum, of similar frequency to radar beam energy, and at a frequency of 2,450 megacycles per second penetrates several centimetres into biological material (Schwan, 1960). This energy is absorbed and converted into heat within the tissue. In effect microwave energy is an alternating electro-magnetic field, the direction of which changes

2,450,000,000 times every second. Dipolar molecules, such as water, present in the field, are forced to oscillate at this frequency and this increases their thermal agitation and generates heat. The amount of heat produced within the tissue can be controlled by adjustment of the energy level and the duration of exposure.

In recent years the development of the magnetron has made available a source of microwave energy that is sturdy, reliable, and relatively inexpensive. Microwave ovens are widely used for the rapid and uniform cooking of steaks and whole chickens. Other applications include food sterilization, grain drying, and the splitting of concrete (Puschner, 1966).

The method described here was developed to determine whether the theoretical possibility of producing histological fixation by microwave heating could be achieved in practice.

Apparatus

The power source is a Microtron 200¹.

SPECIFICATIONS

Microwave generator

Mullard JP2-02 magnetron

Power input

630 watts maximum on A.C. 200/250 volts, 50 cycles

Power output

3-200 watts in two power ranges

Operating frequency

2,450 ± 50 megacycles/second (wavelength 1.25 cm)

Appropriate rectifying valves, delay circuit, fuses, pilot lights, and controls are fitted.

The Microtron 200 was designed principally for use in hospital physiotherapy departments. It can be adapted to 110 volts 60 cycles AC electricity supply. The operating frequency is in one of the bands designated for industrial, scientific, and medical use by the International Radio Conference at Geneva 1959 (GPO 1968).

The microwave chamber designed for histological fixation consists of a section of rectangular copper wave-guide with internal dimensions of 7.2 × 3.2 cm and length 30.2 cm. A sliding door is provided for the insertion of specimens. It closes to seal the chamber during operation and a safety switch cuts off the power supply when the sliding door is open. The specimens rest in the

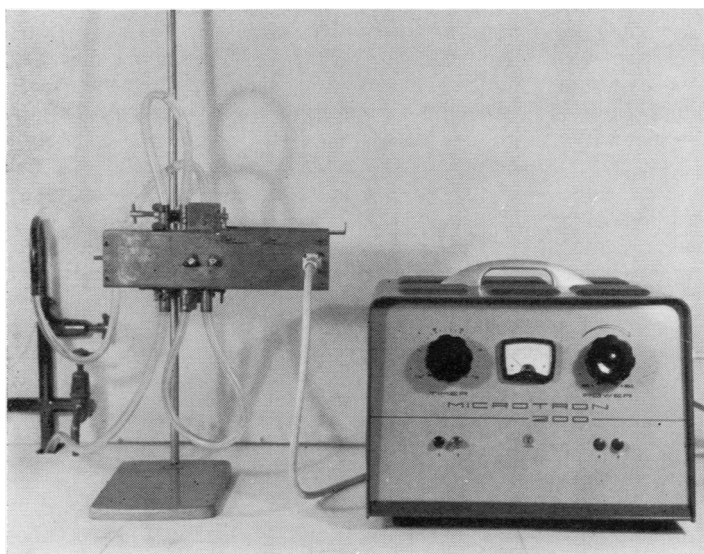


Fig. 1 *The Microtron 200 power source connected by coaxial cable to the microwave chamber.*

chamber on top of an expanded polystyrene block surfaced with a thin sheet of polytetrafluoroethylene. The connexion for the coaxial cable from the Microtron 200 is fitted 2.15 cm from one end of the chamber. A piston-type tuner is placed at the other end of the chamber and two tuning stubs are located in the chamber wall. The permanent settings of the tuning devices have been adjusted by the manufacturer by means of reflected power meter readings. Three loops of polyvinyl chloride tubing contain a constant flow of tap water to constitute a dummy load (Fig. 1).

Materials

The tissues exposed to fixation included fresh postmortem rabbit kidney, liver, lung and muscle; postmortem mouse kidney and liver, and fresh postmortem normal human liver and kidney.

The tissues were trimmed to standard 1.0 cm cubes, using specifically designed two-bladed cutters.

Method

The cubes of tissue were placed in the midline of the chamber with sides parallel to the chamber walls. The optimum energy level and duration of exposure were determined by numerous trials, and 75 watts output for 90 seconds was found suitable for 1 cm cubes of tissue.

Approximate indications of the temperature achieved during exposure were obtained by inserting small slips of thermosensitive wax paper into the tissue cubes before exposure. Accurate determination of the temperature is difficult as metal parts cannot be present within the chamber during exposure.

Subsequent processing of the tissue cubes utilized conventional methods including dehydration, wax embedding under moderate vacuum, section cutting, staining, and mounting under coverslips.

Results

On macroscopic examination after fixation, the tissue specimens were firm but not hard. There was some darkening of the tissue but no loss of macroscopic detail and no appreciable shrinkage.

Kidney tissue is selected for a description of the microscopic appearances as it showed changes representative of all the tissues examined, and as the microscopic appearance of kidney tissue has been advocated by Baker (1958) as a reasonable test of fixation.

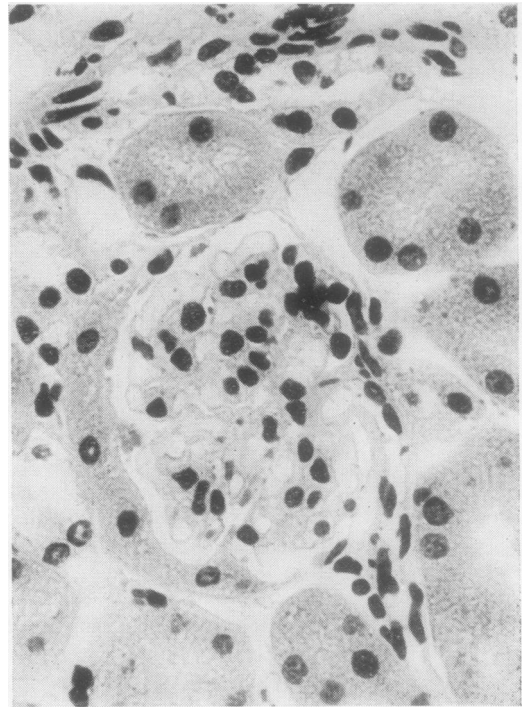


Fig. 2 *Mouse kidney tissue fixed by microwaves ($\times 200$ H & E). Most of the tubules have closed lumina. The capillary loops appear empty of erythrocytes.*

The renal capsule, the glomeruli, the proximal, distal and collecting tubules, and the vascular system were all clearly preserved. Staining was uniform throughout. Shrinkage was slight and the customary artefacts of periglomerular and luminal spaces were slight or absent. Nuclear structures were quite well preserved. Cytoplasmic details were well preserved. The brush border of the tubular epithelial cells was not often seen as most of the tubules remained closed (Fig. 2).

A curious feature was the loss of erythrocytes, and to a lesser extent of the collagen fibres after fixation. The appearance suggested that the red cells had lysed and formed a homogeneous deposit of haemoglobin and stromal protein.

Safety

The main hazard from microwave radiation is heat injury (Seth and Michaelson, 1964). Other possible hazards include the 'specific thermal effect' described by Tomberg (1961) and the production of chromosomal aberrations described by Heller and Teixeira-Pinto (1959). In the apparatus described here, the power level is very low, and even if the safety cut-out failed,

the sensation of heat would warn the operator of danger long before any tissue damage occurred.

Another theoretical danger with some microwave sources such as the klystron is that ionizing radiation may be emanated when the applied voltage exceeds 15 kilovolts (De Minco, 1961). The magnetron described here could here not survive such a voltage.

Comment

The apparatus described was designed to determine whether microwaves could produce fixation and used 1 cm cubes of tissue. In a larger apparatus, larger blocks or slices of tissue, 3 or more cm thick, could be similarly treated, and apparatus could be constructed to process large numbers of specimens using a mechanical feed system.

The main disadvantage of microwave fixation is the unexplained disappearance of erythrocytes, and to a lesser extent of collagen fibres. Further work may show a way to eliminate this defect. The main advantages are that it is a reproducible physical method which produces uniform fixation, with minimum shrinkage, in 90 seconds.

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