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Rapid giant paper sections of lungs

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A detailed account is given of a method of preparing giant paper sections of lung for the day following necropsy or, better and more easily, for the subsequent day. This method represents minor modifications to the highly successful Gough–Wentworth technique by challenging the need for lengthy fixation and embedding procedures, but producing results which are thought to be comparable.

In 1949 Gough and Wentworth published their method of preparing whole lung sections mounted on paper which has since been widely used, contributing substantially to the understanding of lung disease. However, the method as most recently described (Gough, 1968) takes 11 days (Table) before the sections are available. In this paper modifications to the Gough–Wentworth technique are described which permit the production of paper sections routinely within 48 hours of necropsy and within 24 hours if necessary (Figs 1–3).

METHOD

DISTENSION The lung is taken at necropsy, weighed and described, and infused with 10% formalin, via the main bronchus, from a large container until it is apparently fully distended. The bronchus is tied or clamped and the lung is placed in a large bath containing formalin. Closure of the bronchus to retain formalin is essential for early slicing. Serial sections of the whole lung can be made by distending with the 'embedding gelatine' (*vide infra*) solution, freezing overnight at $-25°$ C., and sectioning as described in 'Section Cutting'. If the vessels are injected first with barium-gelatine excellent radiographs can be taken after the lung is frozen.

SLICING THE LUNG The lung is sliced sagittally with a ham knife on a rack, giving slices 1·5 cm. thick. The second or third slice from the hilum is usually selected to give a surface relatively free from hilar structures.

The slicing is done (*a*) after one hour if the section is required within 24 hours, or (*b*) early on the morning following the necropsy if 48-hour sections are required. The lung is firmer and easier to slice at this stage.

EMBEDDING The selected lung slice is squeezed gently in running tap-water to wash out some of the formalin and remove some of the air. Then as much fluid as possible is squeezed gently out before placing it in a metal dish of 'embedding gelatine' solution (*vide infra*) and allowing the solution to be taken up sponge-like by the lung tissue. The remaining embedding gelatine is poured rapidly through a nylon pan scrubber to remove blood clot and a small quantity is returned to the dish. This is put in the deep-freeze to just set, so that the lung surface is flat on the bottom. Then the slice will not float up when more embedding gelatine is added to cover the slice. A wooden chuck is floated on top, and the whole dish is put in the deep-freeze at $-25°$ C. until frozen. This takes about 3–4 hours.

SECTION CUTTING The block is removed from the dish after 3–4 hours in the 24-hour sequence, or more conveniently after lunch in the 48-hour sequence. The block is trimmed and sectioned on a Toledo (Toledo Scale Corp., Ohio) bacon slicer, modified by the addition of a supporting strut. (No large-section microtome has been used and the virtues of a moving blade *versus* a moving block have not been explored.) The exact thickness of the sections is not known but it is thought to be between 400 and 600 microns. Thinner sections are usually obtainable with the longer fixation period. A satisfactory section is regarded as one which is thin enough for white paper to be seen through the alveolar ducts and where the pleural outline is substantially intact. Nevertheless, it is almost impossible on the bacon slicer to avoid cutting the leading edge of the block thicker than the trailing edge.

SECTION HANDLING Each section is caught with the left hand (while the right hand pushes the block) and floated into 10% formalin over a sheet of coarse nylon net. Much of the embedding gelatine floats out or can be gently pulled away. The required number of sections are lifted on the nylon net and washed with a gentle jet of running tap-water. The formalin is changed and the nylon and sections are floated back into it.

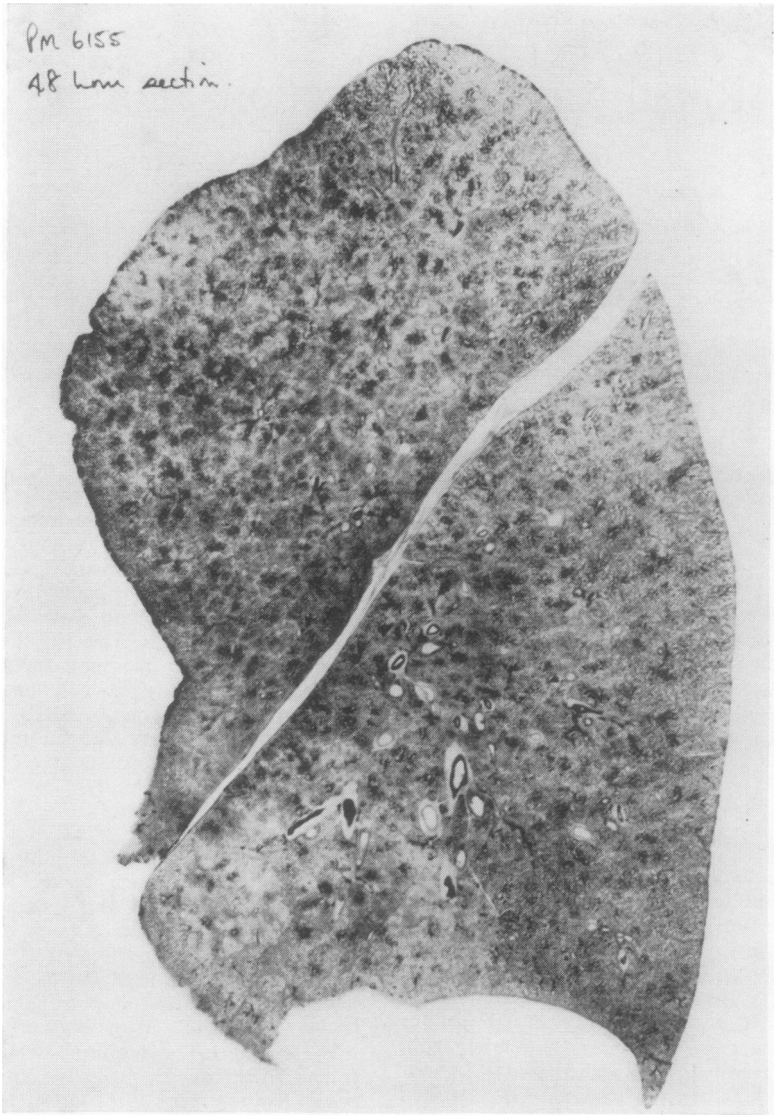

FIG. 1. *A 48-hour section showing marked centrilobular deposition of pigment in a 60-year-old man.*

SECTION MOUNTING No delay occurs after the last stage. A Perspex sheet is flooded with 'mounting gelatine' (*vide infra*) solution. A section picked by hand like a wet cloth from the formalin is put on the Perspex and teased out until it is flat on the gelatine. All bubbles and embedding gelatine are stroked out with a finger. More mounting gelatine is poured on and Whatman's 3 mm. chromatography paper is laid on top. Bubbles are stroked out and the paper is wiped with a sponge. By turning the Perspex over, the section can be gently stroked into the best position as the surplus gelatine solution drains off but care is needed with the one-hour fixed specimen to prevent blood mixing with the mountant in the surround and giving a dirty final background.

DRYING The most rapid method has been to put the Perspex sheets end on to the air-conditioning draught (23° C.). Overnight the section is dry enough to demonstrate. Stripping it off the Perspex requires

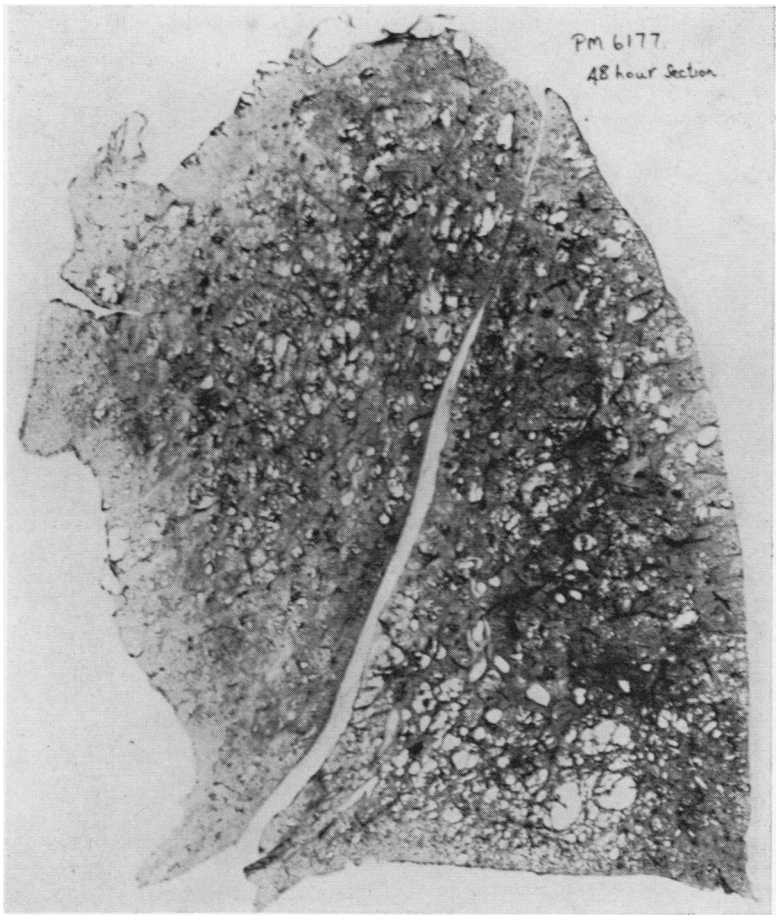

FIG. 2. *A 48-hour section showing extensive centrilobular emphysema, with bullae on the free edges of the upper lobe in a 62-year-old man.*

another hour in the warm cabinet after all damp patches have gone. Then the paper-mounted section strips off easily and should be trimmed to remove surplus gelatine at the edge, which can be sticky.

EMBEDDING GELATINE SOLUTION The formula used is:

Cellosolve	240 ml.	(ethylene glycol monoethyl ether)
Capryl alcohol	30 ml.	
1% Thiomersal	20 ml.	(antiseptic)
Gelatine (80–100 bloom)	1,000 g.	
Water to	5,800 ml.	

(1,000–1,200 ml. are used per block. The solution is kept in the warm cupboard at 40° C. for 3–4 days before use and is used as required after that.)

This is much the same formula as that described by Gough (1968), except that the gelatine used is only two-thirds of that recommended. The frozen gelatine remaining round the cut section is thus more friable and soaks out rapidly into the formalin, leaving a clearer section and allowing penetration of the section by the formalin. Fresh embedding solution is too rubbery when set and does not separate well from the section.

MOUNTING GELATINE SOLUTION The formula recommended by Gough (1968) has been slightly modified:

Glycerine	50 ml.	(70 ml. recommended)
Cellosolve	40 ml.	(ethylene glycol monoethyl ether)
1% Thiomersal	10 ml.	
Gelatine (80–100 bloom)	75 g.	
Water to	1,000 ml.	

(About 700 ml. is used for six sections.)

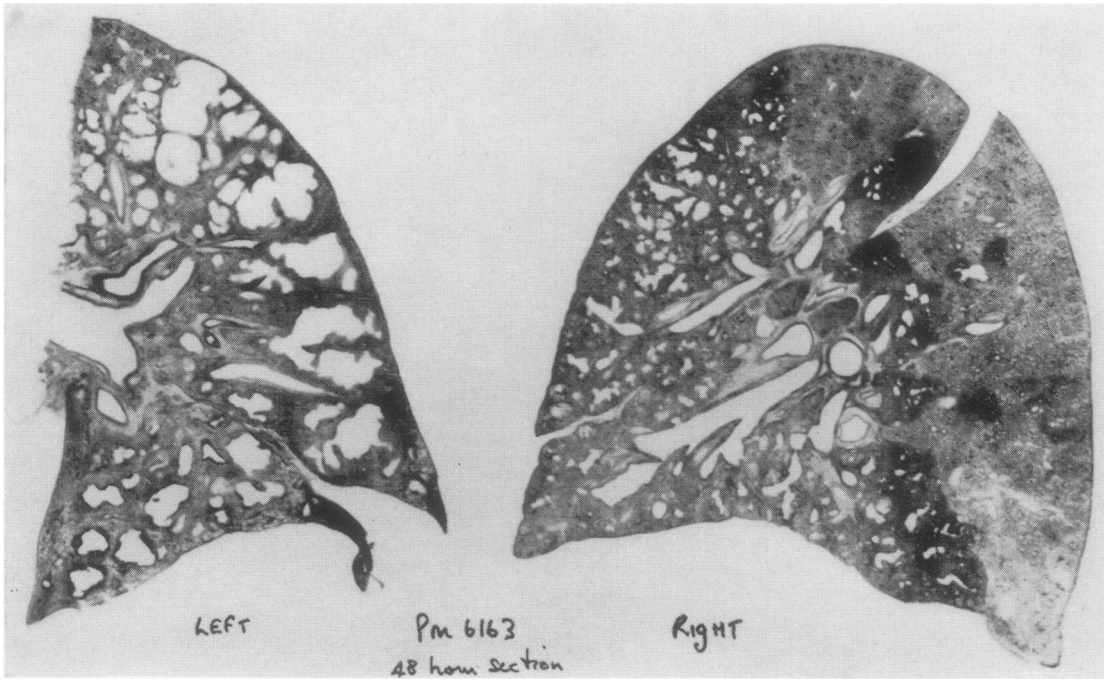

FIG. 3. *A 48-hour section showing bronchiectasis in both lungs in a 4-year-old girl.*

This is made up within 24 hours of use and kept in the warm cupboard (40° C.). This makes the final paper section less sticky and deliquescent in a tropical climate. There has been some problem with moulds growing on the paper sections, but these have been easily wiped off with 98% alcohol, and prevented by keeping them in an air-conditioned room or dry cupboard.

TABLE

METHODS OF PREPARING WHOLE LUNG SECTIONS

	Gough and Wentworth Method	Present Method
Fix whole	Minimum 2 days	(*a*) 1 hour (24-hour method) (*b*) 18–20 hours (48-hour method)
Fix slice	'Few days' (=minimum 2)	—
Wash slice	Minimum 3 days	Squeeze in water
Embed slice ..	Minimum 3 days	Squeeze in embedding solution
Freeze block ..	Several hours	3–4 hours
Section and harden and mount ..	Minimum 1 day	1 hour
Dry	?	18 hours
	11 days	(*a*) 24 hours (*b*) 48 hours

DISCUSSION

The method described produces good results at 24 hours and better results at 48 hours and provides excellent material for demonstrations. No slice takes up laboratory space for more than two days, and the technique can be applied to all necropsies and presumably to resected lungs.[1]

In the original method the lung was fixed whole for a minimum of 2 days, each slice fixed for a minimum of 2 days, and the formalin washed out for a minimum of 3 days. The sections cut were fixed in formalin for a minimum of 24 hours (to harden the gelatine) and washed for another 1–2 hours. This rapid method obtains fixation from the initial formalin distension for a minimum of one hour (although the overnight fixation of 18–20 hours seems more natural to

[1]From the point of view of the operator the 24-hour sequence can be a whole-time job, as he has to find out or be told when lungs to be sectioned are available, distend them, wait an hour, slice and embed them, wait 3–4 hours, and cut and mount them for overnight drying. It is easier to slice and embed one or more better fixed lungs on arrival in the morning and cut and mount them any time in the afternoon.

the pathologist and produces better results), and further fixation when the section is floating in the formalin, and also after mounting, for the formalin is not washed off. It is probably because the penetration required for alveolar and bronchial walls is so small that such fixation is enough, and it is probably desirable on these grounds also to use sections containing few of the thick hilar structures. Reducing fixation and washing in this way saves about 7 days.

A further 2 days is saved by allowing the slice to take up the somewhat less viscous embedding gelatine solution like a sponge in a few minutes as opposed to the histological approach of removing bubbles by vacuum and incubator. No trouble from ice crystals or proteolytic enzymes has been noticed. As long as it is reasonably dry the section can be handed round or projected on the epidiascope before it is stripped off the Perspex, which, in fact, protects it.

The method saves a little gelatine and a lot of valuable time and space. It is very satisfying to have the paper sections while the necropsy is still fresh in the mind, and at demonstrations they are the most convincing evidence of the extent of emphysema or pneumonia or other lesions in that lung. The technique could easily be applied to epidemiological studies.

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REFERENCES

Gough, J. (1968). In *The Lung*, Ed. Liebow, A. A., and Smith, D. E., p. 313. Williams and Wilkins, Baltimore.

—— and Wentworth, J. E. (1949). The use of thin sections of entire organs in morbid anatomical studies. *J. roy. micr. Soc.*, **69**, 231.