Technical methods

Simultaneous demonstration of cyst walls and intracystic bodies of *Pneumocystis carinii* in paraffin embedded lung sections using Gomori's methenamine silver nitrate and Giemsa stain

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Laboratory diagnosis of *Pneumocystis carinii* pneumonia is based on identifying the causative organism in material taken from the patients, and showing the presence of cysts. Among the many staining methods for *P carinii* pneumonia in lung tissue sections, hematoxylin and eosin staining is essential to see host reaction, but it seldom stains the organism itself. On the

other hand, it is well known that Gomori's methenamine silver nitrate and toluidine blue-O are reliable for staining the cyst wall but not for the intracystic body.^{1,2} Therefore it is sometimes difficult to distinguish *P carinii* cysts from the ascospore of fungi that have similar staining properties for Gomori's stain and toluidine blue-O stain. The best method at present for staining the intracystic bodies as well as the trophozoites is to use Giemsa stain, but it only barely stains the cyst wall.³ This study describes an advanced technique that simultaneously shows the cyst walls and intracystic bodies in the tissue sections by using Gomori's stain and Giemsa double staining.

Material and methods

Paraffin embedded lung sections containing *P carinii* were prepared from the following sources: human lung taken from a 6 month old boy who had had congenital immune deficiency syndrome and who had died from *P carinii* pneumonia; Wistar strain rat lungs killed after five to eight weeks of cortisone treatment⁴; BALB/c background nude mice lungs killed at four to six months after being infected with *P carinii* in our laboratory; 5 month old mongrel cat lungs killed after

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Fig 1 (a) Human lung; cyst walls and intracystic bodies of mature cysts (arrows) are satisfactorily stained with Gomori's stain and Giemsa, respectively. Trophozoites are also seen. (b, c). Same visual fields as (a), but focal planes are slightly different. Parenthesis like structure (arrow) is slightly visible in mature cyst in (b), and it becomes more apparent in (c). (Gomori's methenamine silver and Giemsa.) $\times 2000$.

Fig 2 (a, b) Human lung; photomicrographs under slightly different planes of focus of same visual field. Parenthesis like structures (arrowheads) are clearly seen at plane of focus in empty cysts. Parenthesis like structures cannot be seen in same empty cysts viewed at slightly different plane of focus (arrows). (Gomori's methenamine silver and Giemsa.) $\times 2000$.

three weeks of prednisolone acetate (10 mg weekly) treatment.

STAINING PROCEDURE

- 1 The lungs were fixed in 10% formalin, and paraffin sections were prepared in the usual way.
- 2 They were stained with Gomori's stain using Grocott's methenamine silver method.²
- 3 Afterwards they were stained with Giemsa (2 drops for 1 ml of phosphate buffer solution pH 7.2) for one hour.
- 4 They were washed in distilled water for a few seconds.
- 5 They were dehydrated by three changes of acetone as follows: acetone I (10 seconds); acetone II (one minute); acetone III (10 minutes).
- 6 Made clear by three changes of xylene, 10 minutes each, then mounted as usual.

Results

The double staining of *P. carinii* with Gomori's stain and Giemsa, means that the cyst wall and parenthesis like structure stained a brownish black colour with Gomori's stain, and the intracystic bodies and trophozoites stained blue by Giemsa.

Figs 1a–c show the sections of human lung infected with *P. carinii* and stained with Gomori's stain followed by staining with Giemsa.

Figs 2a and b show *P. carinii* obtained from human lung under slightly different planes of focus of the same visual field. A large cluster of *P. carinii* cysts and trophozoites filled an alveolar space. Some of the cysts were considered to be collapsed empty cysts because they were crescent or cup like in shape and had no intracystic bodies inside. Sometimes we found that the cyst wall did not stain with Gomori's stain but was stained slightly blue, probably due to Giemsa. No difference was found between staining properties in *P. carinii* from a human, rats, nude mice and a cat.

Discussion

Gomori's stain and toluidine blue-O were used for the cyst wall staining of *P. carinii*. Gomori's stain, in particular, selectively stains the cyst wall and the so called parenthesis like dark brown structure in strong contrast to the background. On the other hand, Giemsa usually stains intracystic bodies and trophozoites rather than the cyst walls.³ The double staining of *P. carinii* with Gomori's stain and Giemsa successfully stained the cyst wall, the parenthesis like structure,

and the intracystic body at the same time. Trophozoites can also be shown by Giemsa staining.

Kim *et al*⁵ double stained *P. carinii* on impression smears of rat lungs with methenamine silver and polychrome methylene blue and stated that these parenthesis like structures seemed to be part of the cyst wall or at least closely related to the cyst wall. According to our observations, these structures can be mainly seen in empty cysts but sometimes in mature cysts that contain the intracystic bodies. These structures may correspond to internally thickened parts of the cyst walls. We noticed that the cyst wall of some cysts containing intracystic bodies was not stained with Gomori's stain. Although the reason for this was not clear, one possibility is that they are thin walled pneumocysts containing daughter cells, as Vossen *et al*⁶ indicated.

It is well known that the Gomori stain staining technique is hard work: the double staining method proposed here is no less so. As a next step, modifications to speed up Gomori staining should be considered for use.⁷⁻¹¹

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New method for typing coagulase negative staphylococci

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Coagulase negative staphylococci are found as pathogens in an increasing number of infections, usually associated with prosthetic implants and catheters.¹ The importance of isolates of these bacteria is often uncertain because they are also common contaminants. The interpretation of repeated isolates depends on a suitable typing system. Several methods have been used, including biotyping,^{2,3} antibiograms,⁴ phage typing,⁵ plasmid profile and restriction endonuclease analysis.⁶ No one method, however, has proved ideal, and often a combination

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of methods is necessary for epidemiological studies.

We developed a new typing system based on sodium dodecyl sulphate polyacrylamide gel electrophoresis of ³⁵S-methionine labelled bacteria (radio-PAGE). This method was compared with a combination of biotyping and antibiograms using a series of coagulase negative staphylococci isolated from patients with peritonitis who were being treated with continuous ambulatory peritoneal dialysis (CAPD).

Methods

SOURCE OF STRAINS

Forty isolates of coagulase negative staphylococci were obtained from 38 episodes of acute peritonitis from eight patients who were being treated with CAPD.

MICROBIOLOGICAL METHODS

Coagulase negative staphylococci were isolated and