



Title	PRELIMINARY APPLICATION OF A FORMALIN FIXED TISSUE SECTION TO THE INDIRECT FLUORESCENT ANTIBODY TEST AND INTRAOVAL PRECIPITIN REACTION FOR THE DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA
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PRELIMINARY APPLICATION OF A FORMALIN FIXED
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ANTIBODY TEST AND INTRAOVAL PRECIPITIN
REACTION FOR THE DIAGNOSIS OF
SCHISTOSOMIASIS JAPONICA

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A formalin fixed tissue section was applied to the indirect immunofluorescent antibody test (IFAT) and intraoval precipitin (IOP) reaction for the diagnosis of schistosomiasis japonica. The liver tissue of an infected mouse with the Philippine strain of *Schistosoma japonicum* was prepared according to the routine process for histopathological sectioning.

Specific fluorescence was detected in the space between the vitelline membrane and the surface of miracidia in the egg, and it disappeared with periodic acid treatment even though the space had been strongly PAS stained. The formalin fixed tissue section also revealed an IOP reaction in the area where the specific fluorescence was detected. These results indicated that the antigen response to the IFAT and IOP tests corresponded to the responses of heat stable substances such as polysaccharides or glycoproteins.

We suggested that formalin fixed sections are more practicable in IFAT and IOP tests than are cryostat processed tissue sections and lyophilized or fresh whole eggs for the diagnosis of schistosomiasis or other parasitic infections.

INTRODUCTION

The success of the circumoval precipitin (COP) test has been recognized in the immunodiagnosis of *Schistosoma* spp. infections^{1,6,7,11,12,14,16)} and is known to be more sensitive and specific than the complement fixation test, radioimmunoassay, Ouchterlony immunodiffusion and ELISA.^{1,13)} Furthermore, since the COP test has been successfully simplified using lyophilized egg antigens, it has been well employed in field surveys.^{2,10,12,15)}

It is known that the fluorescent antibody technique is also of diagnostic value because of its specificity and sensitivity^{4,5)}; however, as frozen sections may be used in FAT, it is not a practical technique for field surveys of schistosomiasis.

Our recent finding that the lyophilized egg antigens for the COP test were markedly

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heat stable³⁾ led us to consider the application of formalin fixed tissue sections embedded in paraffin wax to IFAT, which will be discussed herein.

MATERIALS AND METHODS

Tissue section: The liver of a mouse (ddY strain) infected with 50 *S. japonicum* cercariae (Philippine strain) was removed at autopsy 7 weeks after the infection and preserved with many egg granulomas in 10 % formalin for one year. The liver tissue was embedded in 56 to 58°C melting point paraffin wax and sectioned into thickness of 3 to 5 μ . After placing the sections on non-autofluorescence slide glasses, the routine procedure of using xylene and ethanol respectively for histopathological section was employed to remove the paraffin and xylene. The sections were washed well with the phosphate buffer solution (PBS; pH 7.2).

Serum: The lyophilized serum (standard serum) of a rabbit was obtained 12 weeks after infection with 600 *S. japonicum* cercariae through skin penetration. The serum was diluted by PBS. FITC-conjugated rabbit anti-IgG, MBL, Japan, lot 099103 (FITC-anti rabbit-IgG) was used in a 1:128 dilution with PBS after determination of the limits of the reaction against undiluted serum.

The COP index calculated by the formula cited by TANAKA (1976) was 20.8 (mean) in the standard serum containing the reaction of the lyophilized eggs purified according to the procedure of KAMIYA et al. (1980).

Incubation: The tissue section with infected serum was incubated in a moisture wet chamber at 37°C for 15 minutes and then washed well with PBS. The section with FITC-anti rabbit-IgG was incubated and then washed with PBS as mentioned above, and then covered with a cover-slip, 18×18 mm in size, sealed with vaseline. The Olympus fluorescence microscope, model BH-RFL, was used for the observation of specific fluorescence, and the photographs were taken by Ektachrome, ASA 200. In addition, PBS and normal rabbit serum were used as controls.

Treatment with periodic acid and sodium metaperiodate: After treatment of the tissue sections with 0.5 % periodic acid and 0.05 M or 0.1 M sodium metaperiodate for 15 minutes, they were washed well with PBS and the same IFAT procedure was carried out.

PAS staining: Routine PAS and hematoxylin staining were employed on the same liver tissue section.

IOP reaction by using the formalin fixed tissue section: The sections and standard serum were the same as those used in the IFA studies. One drop of standard serum was put on the section, and the section was covered with the cover-slip previously mentioned, sealed with vaseline, and incubated in a moisture chamber at 37°C for 48 hours. The Olympus differential interference microscope, model BH-NIC, was used for the observation of precipitin.

RESULTS

Analysis of fluorescence antibody response to egg antigens fixed by formalin: Although the negative controls in the PBS and normal rabbit serum showed no specific fluorescence, the autofluorescence of eggshell colored in orange was detected (figs. 1 & 2).

Undiluted to 1/16 dilution of standard serum: The area between the vitelline membrane and the miracidia and the surface of miracidia and its canal system were also strongly stained greenish yellow.

1/32 to 1/128 dilution: The specific fluorescence in the space under the vitelline membrane (SF) became clear. The fluorescence on the miracidia weakened and disappeared.

1/256 to 1/2048 dilution: The SF was still obvious (figs. 3 & 4).

1/4096 to 1/32768 dilution: Although the eggs without SF appeared, some of the eggs showed weak SF (figs. 5 & 6).

1/65536 to 1/131072 dilution: SF became paler, finer, and sometimes discontinuous, although it was still significant for diagnostic purposes.

1/262144 dilution: SF disappeared.

Periodic acid treatment: Specific fluorescence completely vanished, and autofluorescence was observed in the eggshell as shown in the negative control (fig. 7).

PAS staining: The PAS positive area corresponded with that of specific fluorescence observed in the IFAT. The area between the vitelline membrane and miracidia was stained most strongly (figs. 8-11).

IOP reaction by using formalin fixed tissue section: Tiny but obvious precipitins were observed in the space between the vitelline membrane and the miracidia in the egg (fig. 12). No intraoval precipitin reaction was observed in the PBS and normal rabbit serum (figs. 13 & 14).

DISCUSSION

The COP reaction is well adapted to the diagnosis of schistosomiasis,^{2,10,12,15)} and the COP test is of great diagnostic value because its resulting reaction is more sensitive and specific than ELISA, radioimmunoassay, immunodiffusion and complement fixation test.^{1,13)} On the other hand, the fluorescence antibody technique is also a good diagnostic tool for schistosomiasis^{4,5)} because of its sensitivity and specificity; however, the FAT has not been successfully simplified for use in helminthic infections since its procedure is much more complicated than the COP reaction in regard to the preservation of antigens. Recently, we found that the heat stable nature of the egg antigens of *S. japonicum* was responsible for the COP reaction³⁾, which stimulated us to apply formalin fixed tissue sections embedded in paraffin wax to the FAT. This subsequent study

supported the idea, because specific fluorescence was detected in the area between the vitelline membrane and the miracidia. The antigens responded to the fluorescence antibody were also strongly stained by PAS and the treatment with periodic acid caused the complete disappearance of its specific fluorescence. Our findings indicated that the antigens which responded to COP, IOP, and the present IFA corresponded to heat stable substances such as polysaccharides or glycoproteins.^{3,7-9)}

The present IFAT and IOP reactions using the formalin fixed tissue section have the advantages of short incubation time, high sensitivity, and minimal expense for the preparation of the antigen for the diagnosis of schistosomiasis japonica, as compared to the COP test using whole fresh or lyophilized eggs.

These results suggested that the technique using formalin fixed tissue sections in IFAT or IOP reactions for schistosomiasis japonica should be extended to the immunodiagnosis of other parasitic infections.

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EXPLANATION OF PLATES

PLATE I

Figs. 1-7 show the results of IFA studies. $\times 900$ except fig. 3

- Fig. 1 Autofluorescence of the eggshell in the section incubated in PBS
- Fig. 2 Autofluorescence of the eggshell; incubated in normal rabbit serum
- Fig. 3 Specific fluorescence; incubated in the infected rabbit serum diluted at 1:10
- Fig. 4 Specific fluorescence in the space between the autofluorescent eggshell and the surface of miracidia in the infected serum diluted at 1:1024
- Fig. 5 Specific fluorescence; incubated in the serum diluted at 1:16834
- Fig. 6 Specific fluorescence still retaining diagnostic characteristics; incubated in the serum diluted at 1:32768
- Fig. 7 Disappearance of specific fluorescence after treatment with 0.5% periodic acid

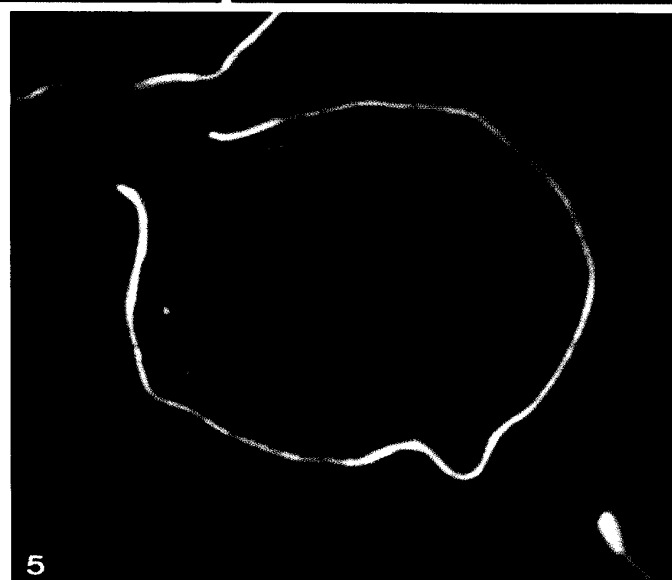
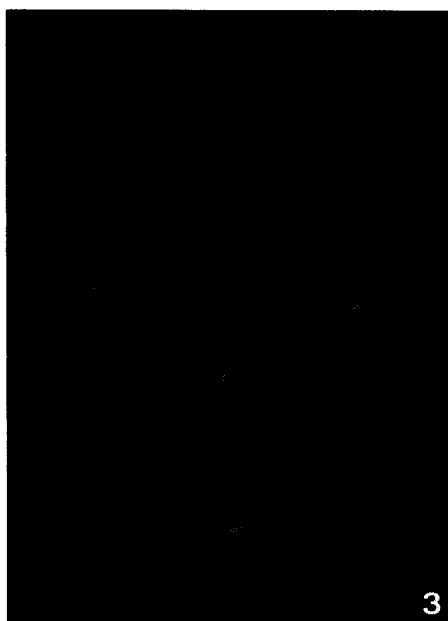
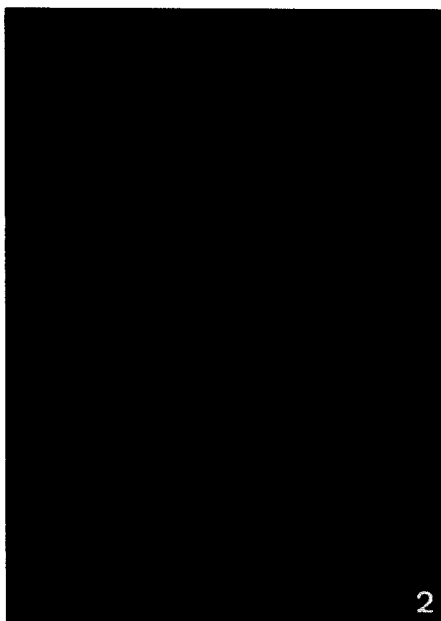
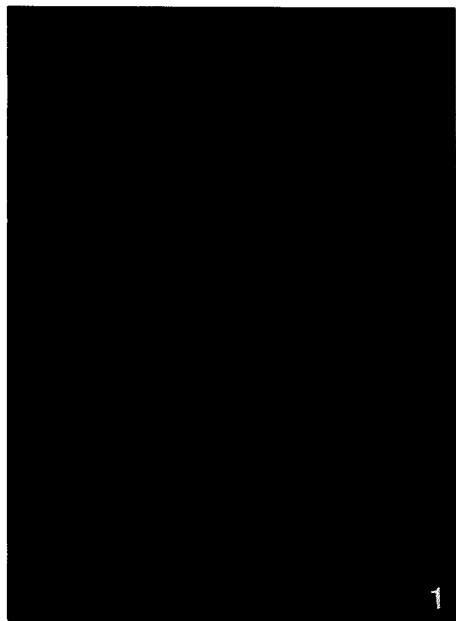


PLATE II

- Fig. 8 PAS positive area between the eggshell and the miracidia $\times 500$
- Fig. 9 Strongly PAS positive area between the eggshell and the miracidia, and the inside of the canal system stained with PAS $\times 900$
- Fig. 10 The area between the eggshell and the miracidium slightly stained with eosin (†) Hematoxylin-eosin stain $\times 500$
- Fig. 11 The material slightly stained with eosin under the eggshell around the miracidia (†) Hematoxylin-eosin stain $\times 900$
- Fig. 12 Intraoval precipitins (IOP; †) of the space between the vitelline membrane and the miracidium, stained by IFAT $\times 540$
- Fig. 13 No intraoval precipitin reaction; incubated in PBS $\times 540$
- Fig. 14 No intraoval precipitin reaction; incubated in normal rabbit serum $\times 540$

