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Technique of the Intraoval Precipitin (IOP) Reaction by Using Formalin Fixed Tissue Section for the Diagnosis of Schistosomiasis

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Circumoval precipitin (COP) test used in the immunodiagnosis of schistosomiasis (Yokogawa *et al.*, 1967; Yogore *et al.*, 1968; Noseñas *et al.*, 1975; Tanaka *et al.*, 1975; Matsuda *et al.*, 1977; Yogore *et al.*, 1979) was stressed to be the most sensitive and specific reaction (Hillyer *et al.*, 1979). Recently, it was found that the egg antigens involved in COP reaction is markedly heat stable and sometimes precipitins in the eggs were observed (Kamiya, 1980). This property of the egg antigens was successfully applyed to the indirect fluorescent antibody technique (IFAT) for the diagnosis of schistosomiasis japonica by using formalin fixed tissue sections of the mouse infected with *Schistosoma japonicum*; moreover, this result induced an idea of applying the formalin fixed tissue section embedded in paraffine wax to precipitin reaction as a new diagnostic tool (Kamiya and Kamiya, 1980). The technique of intraoval precipitin reaction was shown herein.

Materials and Methods

The liver of a mouse (ddY strain) infected with 50 *S. japonicum* cercariae (Philippine strain) was used. The mouse liver with many egg granulomas was preserved in 10% formalin solution for a year. The liver tissue embedded in 56 to 58 C melting point paraffine wax was sectioned into 5 to 10 μm thickness. These tissue sections were kept in the laboratory for 6 months. The routine procedure for the pathological tissue section was employed to get rid of the paraffine and xylene by using xylene and ethanol, respectively. This was followed by washing well with phosphate buffer solution (PBS; pH 7.2).

Serum: The lyophilized serum (standard serum) of a rabbit 12 weeks after the infection of 600 *S. japonicum* cercariae by skin penetration was employed. The standard serum was resuspended in the same volume before lyophilization by adding 0.85% sodume chloride solution. Normal rabbit serum and PBS was used as negative controls.

Incubation: One drop of standard serum was put on the section, covered with a cover-slipe of 18×18 mm in size, sealed with vaseline and incubated in a moisture chamber at 37 C for 48 hours.

Observation: The Olympus differential interference microscope, model BH-NIC, and ordinary light microscope were employed.

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Results

Many minute or short filamented precipitins were clearly observed between the vitelline membrane and the miracidia without staining with FA or PAS by the differential interference microscope (Figs. 1, 3, 5).

The precipitin was also detected by ordinary light microscope (Figs. 2, 4). However, minute precipitins could be more clearly seen by using the differential interference microscope.

No precipitin was observed in the eggs incubated with PBS and normal rabbit serum (Figs. 6, 7).

Discussion

Recently, it was suggested that the egg antigens involved in COPT or indirect fluorescent antibody technique by using the formalin fixed tissue section contain heat stable substances such as polysaccaride or glycoprotein (Kamiya, 1980; Kamiya and Kamiya, 1980; Ohashi and Ishii, 1980).

This suggested the possibility of applying the formalin fixed tissue section of infected animal with *Schistosoma* spp. to precipitin reaction (Kamiya and Kamiya, 1980).

Since the discovery of COP reaction (Oliver-González, 1954), only the lyophilized or fresh eggs were used in the COPT (Rivera de Sala *et al.*, 1962; Yokogawa *et al.*, 1967; Yogore *et al.*, 1968; Noseñas *et al.*, 1975; Tanaka *et al.*, 1975; Matsuda *et al.*, 1977; Hillyer *et al.*, 1979; Yogore *et al.*, 1979). However, purification procedure of eggs for COPT is somewhat complicated and expensive (Kamiya *et al.*, 1980). Therefore, this intraoval precipitin (IOP) reaction by using the formalin fixed tissue section has an advantage of the low cost involved in the preparation of the antigen (formalin fixed tissue section) for the diagnosis of schistosomiasis, as compared with the COPT which requires the use of fresh or lyophilized eggs.

The technique of IOP reaction might be developed as a new diagnostic tool in schistosomiasis. Furthermore, in order to define the minute precipitins, the use of differential interference microscope is strongly recommended.

Summary

The principle of circumoval precipitin reaction was applied to the formalin fixed liver section with egg granuloma of schistosomiasis japonica (Philippine strain).

Intraoval precipitin (IOP) formation was detected in the space between the vitelline membrane and the surface of miracidia in the egg, of which the antibody binding site was stained with IFAT or PAS as shown in my previous work. The technique of intraoval precipitin (IOP) reaction observed without staining has the advantage of requiring only a small expence in the preparation of the antigen (formalin fixed tissue section) for the diagnosis of schistosomiasis japonica, comparing with the COP test which uses the fresh or lyophilized eggs. And also, the differential interference microscope is preferable for the observation of IOP or COP reactions in schistosomiasis.

Present results suggest that the employment of the formalin fixed tissue section in immunological diagnosis of other parasitic infections should be considered.

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ホルマリン固定組織切片を用いた住血吸虫症診断用卵内沈降反応 (Intraoval Precipitin Reaction)

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最近,卵周囲沈降反応 (COP) に関与する抗原が非常に強い耐熱性であることが明らかにされた．この特性に着目して，ホルマリン固定組織切片を用いての沈降反応—卵内沈降反応 (Intraoval Precipitin Reaction) を実施し，特にその手技に関しての検討を行った．

約1年間 10% ホルマリン液中で保存した，フィリピン株の日本住血吸虫感染後7週の，多数の虫卵結節を有するマウス肝臓を，通常の病理組織切片作製法にしたがって，パラフィン包埋，薄切，脱パラフィンを施して使用した．切片は薄切後6カ月間室温に保存したものを用いた．脱パラフィン後，PBS でよく洗い，感染後12週目のウサギ血清を加え，カバーグラスをかけ，周囲をワセリンで封入し，37 C の湿潤箱中で 48 時間反応後，沈降物の形成の有無を判定した．

感染血清と反応させたものでは卵膜 (vitelline membrane) とミラシジウムの間に小さい滴状あるいはフィラメント状の沈降物が認められ，通常の光学顕微鏡でも識別されたが (Figs. 2, 4)，微分干渉顕微鏡観察でより明瞭であった (Figs. 1, 3, 5)．対照として，PBS，ウサギ正常血清と反応させたものでは沈降物は認められなかった (Figs. 6, 7)．

今回の結果から，ホルマリン固定組織切片を用いた卵内沈降反応の技法は，簡便で，安価な住血吸虫症の診断法として利用出来ることが明らかとなった．また，COP，IOP の観察には，微分干渉顕微鏡が有効であることが示唆された．

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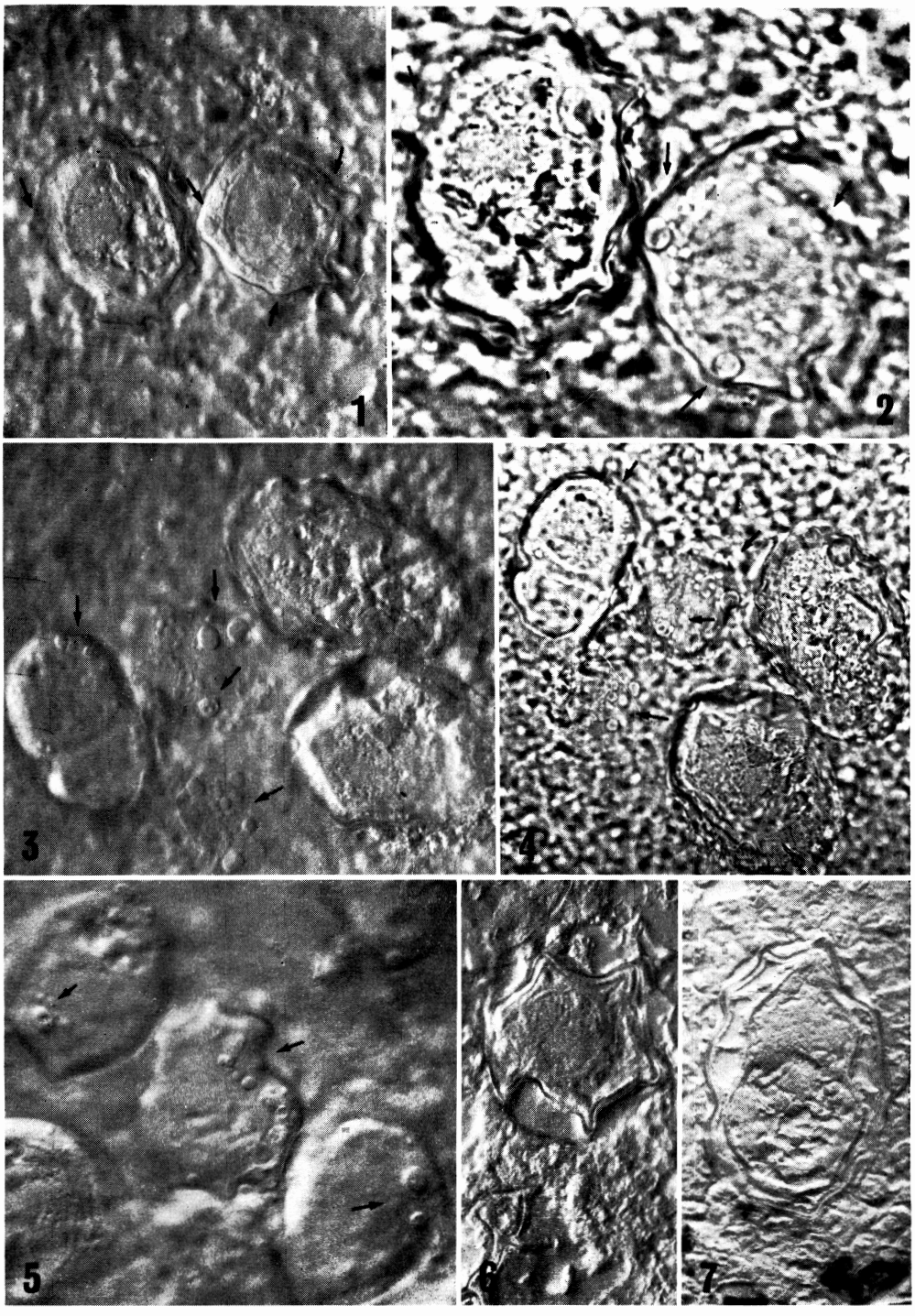
Explanation of Plate

Plate Figs. 1, 3, 5–7 were observed by the differential interference microscope. ×540.
Fig. 1 Many minute precipitins in the space between the vitelline membrane and the miracidia (↗), incubated with infected serum.
Fig. 2 Same precipitins of Fig. 1 by ordinary light microscope (↗), incubated with infected serum. ×880.
Fig. 3 Many precipitins in the space between the vitelline membrane and the miracidia (↗), incubated with infected serum.
Fig. 4 Same precipitins of Fig. 3 by ordinary light microscope (↗), ×480.
Fig. 5 Many minute or filamented precipitins in the space between the vitelline membrane and the miracidia (↗), incubated with infected serum.
Fig. 6 No precipitin, incubated with PBS.
Fig. 7 No precipitin, incubated with normal rabbit serum.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