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Immunodiagnosis of Trichinosis: An Application of Air-Dried *Trichinella spiralis* Muscle Larvae in the Microprecipitin Test

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Trichinosis is widely distributed in the world, including Japan (Yamaguchi *et al.*, 1975, 1982; Ohbayashi and Yamaguchi, 1980; Tebayashi *et al.*, 1981). Immunodiagnostic tests for trichinosis have been tried indirect fluorescent antibody, bentonite floculation, latex agglutination, enzyme linked immunosorbent assay, microprecipitin and many others (Kagan and Norman, 1970; Kagan, 1981). The microprecipitin test reported by Oliver-González (1940) and Mauss (1940) is the most simple and useful diagnostic tool in an endemic area where laboratory facilities are insufficient and the necessary biochemical reagents are unabailable. Kagan and Norman (1970) recommended the microprecipitin test because of its sensitivity and its long use in some diagnostic laboratories. However, they also noted that the need for a supply of living larvae for the procedure reduces its practicality in laboratories where tests for the diagnosis of trichinosis are performed only occasionally. The introduction of lyophilized larvae for the microprecipitin test by Negru *et al.* (1971) is a partial solution to this problem. On the other hand, lyophilized larvae are not always obtained in an endemic area where it is most needed. The purpose of this study was to investigate the use of air-dried *Trichinella spiralis* larvae in the microprecipitin test.

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Materials and Methods

Preparation of *T. spiralis* muscle larvae

The strain of *T. spiralis* isolated from a polar bear, *Thalarctos martimus,* and maintained in experimental animals for nearly 15 years, was used in this study. The larvae were recovered from mice (ddY strain) inoculated previously with 400 *T. spiralis* larvae. The encysted larvae were freed from muscle by the digestion method using 0.2% pepsin and 0.2% HCl solution. Undigested materials were removed by sieving and the larvae were washed several times by sedimentation in physiological saline and then in distilled water. A drop of the suspension containing about 50 larvae was placed on a glass slide. After absorbing the distilled water with filter paper, the slides were dried in drying ovens set at 27, 37 and 60 C for 2 days. The dried larvae, which had adhered to the slides, were preserved with silica gel and stored in a refrigerator at 4 C for more than one year prior to use. Larvae dried at 4 C and at room temperature, as well as those lyophi-

lized or frozen at −80 C, were used for comparing in reactivity in the microprecipitin test.

Serum

Sera collected from 8 patients who had eaten *T. spiralis* infected brown bear meat 1.5–2 months before in Sapporo, Japan, were used. These sera were positive in the microprecipitin test using live larvae of *T. spiralis*. Negative control sera were obtained from 20 healthy persons in an area of Japan where trichinosis is not endemic.

Procedure for the microprecipitin test

Serum samples of 0.05 ml were placed onto the larvae which attached to the glass slide. After mixing the larvae and the serum with a toothpick, a cover slip (18× 18 mm) was placed over the suspension and sealed with vaseline. The slides were then incubated in a moist chamber at 37 C for 24 hours.

Detection of antigens from air-dried *T. spiralis* larvae by the Ouchterlony method

Larvae recovered from C57BL-b^g/b^g mice, 2 months after inoculation, were dried at 4, 27, 37, 60 C and at room temperature for one day. These larvae were then homogenized with a teflon homogenizer in phosphate buffered saline (PBS; pH 7.2). Detection of the antigens was carried out in 1% agarose gel by the double diffusion method of Ouchterlony. A patient serum was used the same as used in microprecipitin test.

Indirect fluorescent antibody test (IFAT)

The air-dried larvae that were positive in the microprecipitin test were washed gently with PBS. The larvae were incubated with 1: 32 dilution of FITC-anti-human IgG at 37 C for 30 minutes. After the incubation, the larvae were washed thoroughly with PBS and mounted in non-fluorescent buffered glycerine. The larvae were then examined under the Olympus fluorescence microscope. IFAT using the air-dried larvae incubated in normal human serum was also carried out.

Results

Microprecipitin test

Larvae dried at 4, 27, 37, 60 C and at room temperature were positive for the microprecipitin test with sera from all of the 8 patients. Gross- and microprecipitates were formed on the larval surface without any specific pattern and its size was various (Figs. 1, 2). Grossprecipitates were also frequently observed at the extremities of fragmented larvae (Fig. 3). In contrast, filamentous precipitates were frequently seen at the orifice and at the esophageal region of larvae preserved at −80 C (Fig. 4). Lyophilized larvae also showed the same precipitates as those of air-dried larvae (Fig. 5). Precipitates on air-dried and lyophilized larvae had the same characteristic morphologically. No precipitate was formed on the air-dried larvae incubated with the normal human sera.

Detection of antigen by the Ouchterlony method

The double diffusion method demonstrated the presence of two distinct precipitin bands when the patient's serum was diffused against the PBS-extract from larvae dried at 4, 27, 37, 60 C and at room temperature and of those frozen at −80 C, although one precipitin band disappeared when tested against the extraction from larvae dried at 60 C (Fig. 6).

Indirect fluorescent study

Specific fluorescence in IFAT study was detected at the precipitates of air-dried larvae incubated with the patient's serum, although no fluorescence was observed on the air-dried larvae incubated in normal

human serum (Fig. 7).

Discussion

The microprecipitin test has the advantage of being carried out anywhere as long as larvae are available. However, as noted by Kagan and Norman (1970), the use of living larvae in the test reduces its practicality for the diagnosis of trichinosis. Subsequently, lyophilized larvae were used in the microprecipitin test (Negru *et al.*, 1971), although lyophilized larvae are not readily available everywhere in endemic areas of underdeveloped countries.

The different size of gross- and microprecipitates was observed on the air-dried and lyophilized larvae under the light microscope in the present studies. However, Kim and Ledbetter (1981) reported the other kinds of microprecipitates, which could be observed by the scanning electron microscope, along the entire surface on living larvae and adults incubated in the immune serum. These different pattern of precipitates might depend on the different components of antigen reacted in precipitin test between the air-dried and the living larvae. It is supposed that the somatic antigens, not the cuticle antigen mentioned by Kim and Ledbetter (1981), has strong reactivity in the precipitin reaction. It is also supported by the present findings that the grossprecipitates were observed at the extremities of fragmented air-dried larvae. The different size of precipitates is presumably due to the size of breakage of larval cuticle, of which the antigens leak out.

It is clear from the present studies that the antigens of air-dried larvae are somewhat heat-stable and retain well its reactivity for the microprecipitin test even after more than one year's preservation at 4 C, although one precipitin band disappeared when tested against the extract from larvae dried at 60 C in the double diffusion method. Furthermore, these precipitates of air-dried larvae incubated in patient's serum were stained with FITC-anti-human IgG, although no fluorescence on the air-dried larvae incubated in the normal human serum was detected. These findings suggest that the microprecipitin test, which is known to be specific and sensitive (Kagan and Norman, 1970; Lamina, 1970), should become more practical with the use of air-dried larvae.

It is concluded that the microprecipitin test using air-dried *T. spiralis* muscle larvae, although time consuming, is possible as diagnostic method, especially in endemic areas of underdeveloped countries where laboratory facilities and biochemical reagents for immunodiagnosis are insufficient.

Summary

Microprecipitin reaction using air-dried *Trichinella spiralis* muscle larvae was investigated. The larvae used were dried at 27, 37, and 60 C for 2 days and then preserved at 4 C in a refrigerator for more than one year. Larvae dried at 4 C and at room temperature were also used. All air-dried larvae showed positive microprecipitin reaction, as did the larvae lyophilized with sera of all 8 trichinosis patients. Gross- and microprecipitates were formed on the surface of air-dried larvae without any specific pattern and its size was various. Grossprecipitates were frequently observed at the extremities of fragmented air-dried or lyophilized larvae. In contrast, filamentous precipitates were frequently seen at the orifice and at the esophageal region of larvae preserved at −80 C. The microprecipitin reation was not observed with control sera of 20 healthy persons. The precipitates on air-dried larvae were stained by FITC-anti-human IgG in indirect fluorescent antibody study. Furthermore, soluble antigens extracted from the larvae dried at 4, 27, 37, 60 C and at room temperature

were detected by the double immunodiffusion method of Ouchterlony. These findings suggest that the microprecipitin test using air-dried *T. spiralis* muscle larvae can be of practical use in the immunodiagnosis of trichinosis in the endemic area of underdeveloped countries.

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自然乾燥筋肉内旋毛虫幼虫を用いた沈降物形成反応（microprecipitin test）の検討

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旋毛虫症の免疫診断法の一つである沈降物形成反応(microprecipitin test)の簡便化の試みとして，自然乾燥した旋毛虫幼虫の本法への応用を検討した．感染マウス筋肉を消化して得た幼虫をスライドグラスにはりつけ，27, 37, 60C で2日間乾燥させた後，約1年間4C で保存した虫体，ならびに4C あるいは室温で乾燥させた虫体を使用した．これらの虫体に，1980年，札幌で発生した旋毛虫症患者血清を乗せ，乾燥を防ぎ，37C で24時間反応させ，沈降物形成の有無を判定した．いずれの自然乾燥虫体も，患者血清8例全例との間に凍結乾燥虫体と同様の明瞭な沈降物の形成が認められた．特に，破損した虫体の断端に顕著な沈降物が出現した．一方，対照群の健康人20例の血清との間に沈降物の形成は認められなかった．また，これら自然乾燥虫体より抽出した抗原はゲル内沈降反応により，患者血清との間に沈降線が形成され，60C 乾燥虫体抗原以外は，−80C 凍結虫体抗原と同様な抗原性を保有していることが明らかになった．また，沈降物を形成した自然乾燥虫体を用いて，間接螢光抗体法を実施したところ，沈降物に特異螢光が認められた。今回の成績から，自然乾燥旋毛虫幼虫が旋毛虫症の沈降物形成反応に応用出来ることが明らかになった．したがって，本法の場合，通常生きた幼虫を用意しなければならないことが指摘されていた点，ならびに，生きた虫体を使用した場合，形成された沈降物が虫体より離脱し，判定に困難を来す点など，これらの問題点を除去出来る利点がある．自然乾燥虫体の本法への応用により，実験設備，生物化学製剤等の供給の充分でない開発途上国の本症の流行地での簡便な免疫診断法として，沈降物形成反応を利用出来ることが示唆された．

Explanation of Figures

Fig. 1 Precipitates on larvae air-dried at 27 C (arrows). ×300.

Fig. 2 Precipitates on larva air-dried at room temperature. ×200.

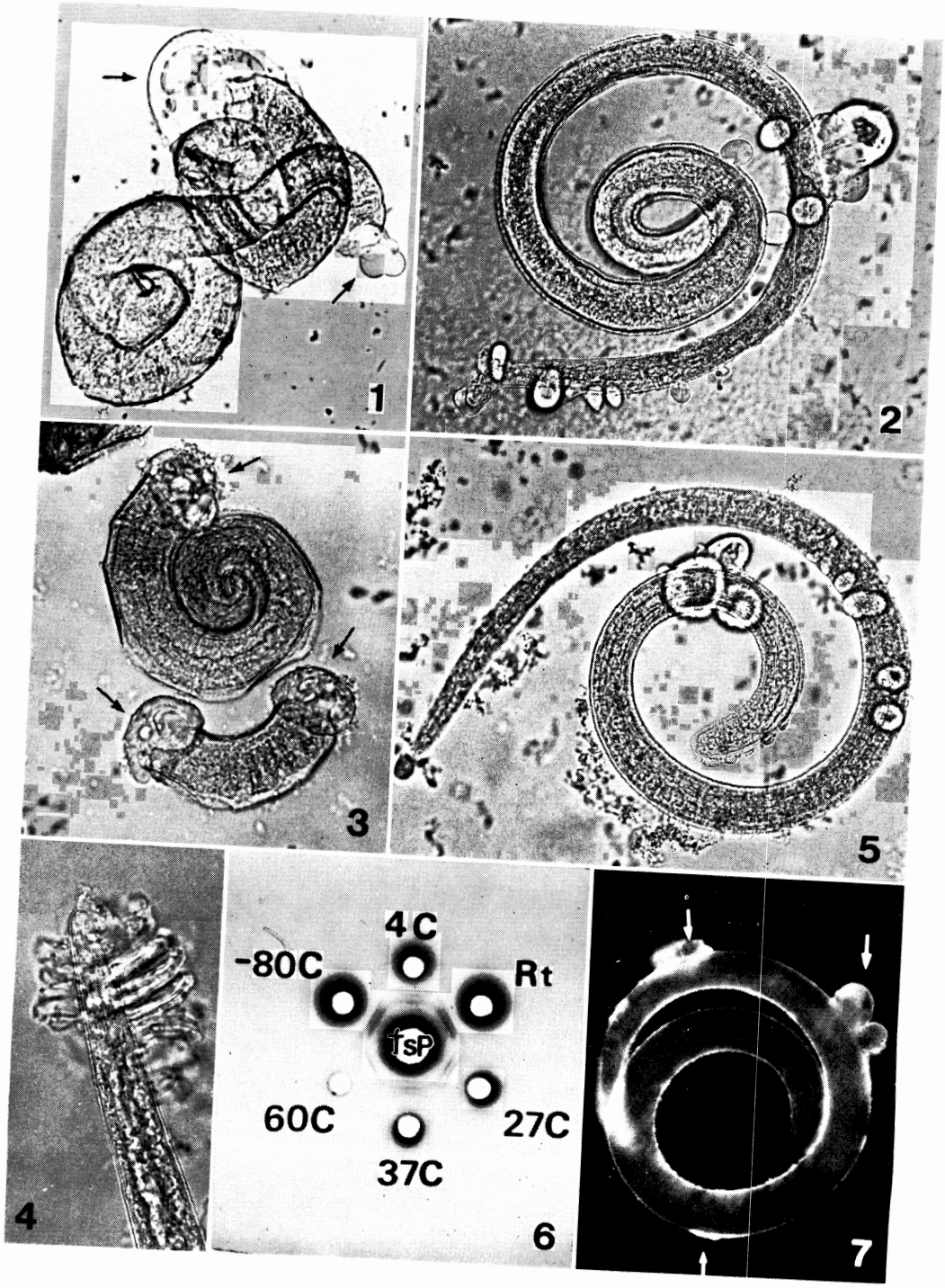
Fig. 3 Distinct precipitates at the extremities of fragmented larva air-dried at 60 C (arrows). ×300.

Fig. 4 Filamentous precipitates at the esophageal region of larva preserved at −80 C. ×400.

Fig. 5 Precipitates on lyophilized larva. ×200.

Fig. 6 Immunodiffusion plate demonstrating the presence of two distinct precipitin bands when the patient's serum (TsP) was diffused against the extract from larvae dried at 4, 27, 37, 60 C and at room temperature (Rt) and frozen at −80 C. One precipitin band was absent against the extract from larvae air-dried at 60 C.

Fig. 7 Specific fluorescence stained with FITC-anti-human IgG showing precipitates on air-dried larvae (arrows). ×200.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2
3
4
5
6
7
4C
-80C
Rt
fsP
60C
27C
37C