protein and 8μg of the purified protein in each tube). Electrophoresis was carried out at 1.5mA per tube at stacking gel and 3mA at separating gel. Gels were stained with 0.125% Coomassie brilliant blue R-250.

SDS-PAGE of Laemmli (1970) was adopted in reducing condition to observe the subunits and measure the molecular mass of these proteins. Stacking gel of 3% and 10~15% linear gradient separating gel was used. Samples were heated at 95°C for 5 minutes with the same amount of sample buffer containing 10% 2-mercaptoethanol.

SDS-PAGE/immunoblot was done as described by Tsang *et al.* (1983). After transfer of the separated proteins to nitrocellulose paper, MAbs were reacted for an hour. Then 1 : 500 diluted peroxidase conjugated anti-mouse IgG goat IgG (Cappel, USA) was reacted. Finally substrate containing 3% H_2O_2 and 0.02% 3,3′-diaminobenzine was reacted.

5. **Immunohistochemical localization of the purified proteins**

Paraffin blocks of a cat lung cyst containing 13-week old *P. westermani* were cut and deparaffinized. The sections were equilibriated with PBS (0.1M, pH7.6). Endogenous peroxidase activity was inhibited by treating the slides in 2% H_2O_2 for 5 minutes at 36°C. MAbs diluted in 1 : 50 in PBS were reacted for 30min. at 36°C. After rinsing, the slides were reacted with biotinylated seconary antibody for 30min at 36°C. Peroxidase complex was reacted for 20min. at 36°C. The reaction was colored with AEC (3-amino, 9-ethylcarbazole) chromogen. Finally, the sections were counterstained with water-based hematoxylin. In addition to the MAbs, a confirmed paragonimiasis serum was reacted as a primary antibody to compare the stainability of polyclonal antibody with those of Mabs.

RESULTS

1. Monoclonal antibodies and immunoaffinity chromatography

Fig. 1 showed SDS-PAGE/immunoblot findings

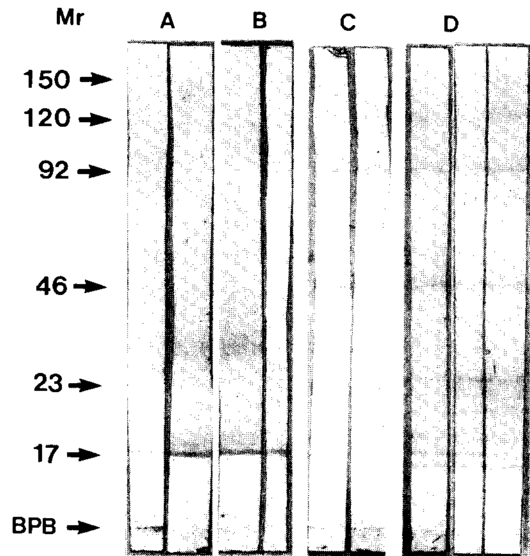


Fig. 1. Findings of SDS-PAGE/immunoblot of MAbs reacted to crude saline extract of adult *P. westermani*.
A: PFCK-21, B: PFCK-44,
C: PFCK-136, D: PFCK-189

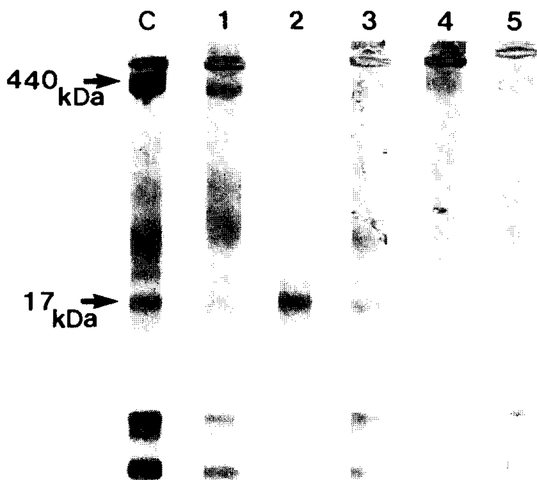


Fig. 2. Findings in disc-PAGE of the component proteins of adult *P. westermani* obtained in affinity chromatography.
C: Crude extract, 1: Unbound protein(UP) from affinity chromatography using PFCK-44, 2: Bound protein(BP) of PFCK-44, 3: UP of PFCK-136, 4: BP of PFCK-136, 5: UP of PFCK-44 and PFCK-136

of MAb reacting to the component proteins in the crude extract. MAbs of PFCK-21 and PFCK-44 series showed the reactions to 17 kDa only (Lanes A and B). PFCK-136 series showed the reactions to 23, 46 and 92 kDa bands (Lane C). MAb of PFCK-189 series showed the reactions to 16, 17, 23, 46, 92, 120 and 150 kDa bands (Lane D). This series of MAb were discarded.

Immunoaffinity chromatography using PFCK-44 and PFCK-136 MAbs as ligands was done. The affinity purified protein using PFCK-44 as a ligand was shown to be band 4 in disc-PAGE of the crude saline extract (Fig. 2, lane 2). On the contrary, when PFCK-136 was used as a ligand, band 1 protein was purified (Fig. 2, lane 4). Unbound proteins to PFCK-44 and PFCK-136 revealed decreased stainability at the corresponding bands (Fig. 2, lane 5).

Fig. 3 exhibited SDS-PAGE findings of the purified proteins. The protein purified by PFCK-44 showed a single SDS-polypeptide band of 17 kDa in reducing condition (lane 2). Another purified protein by PFCK-136 was 23 kDa in molecular mass (lane 5).

2. Localization of the purified proteins

Fig. 4 showed a section of adult *Paragonimus*

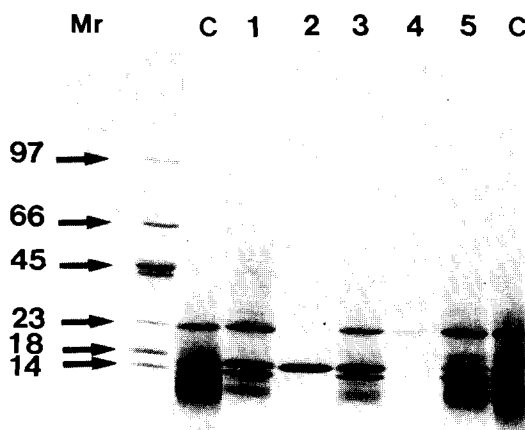


Fig. 3. SDS-PAGE findings of the component and purified proteins of *P. westermani* in 10~15% gradient gel.
Mr: Molecular mass in kDa, C: Crude extract, 1: UP of PFCK-44, 2: BP of PFCK-44, 3: UP of PFCK-136, 4: BP of PFCK-136, 5: UP of PFCK-44 and PFCK-136

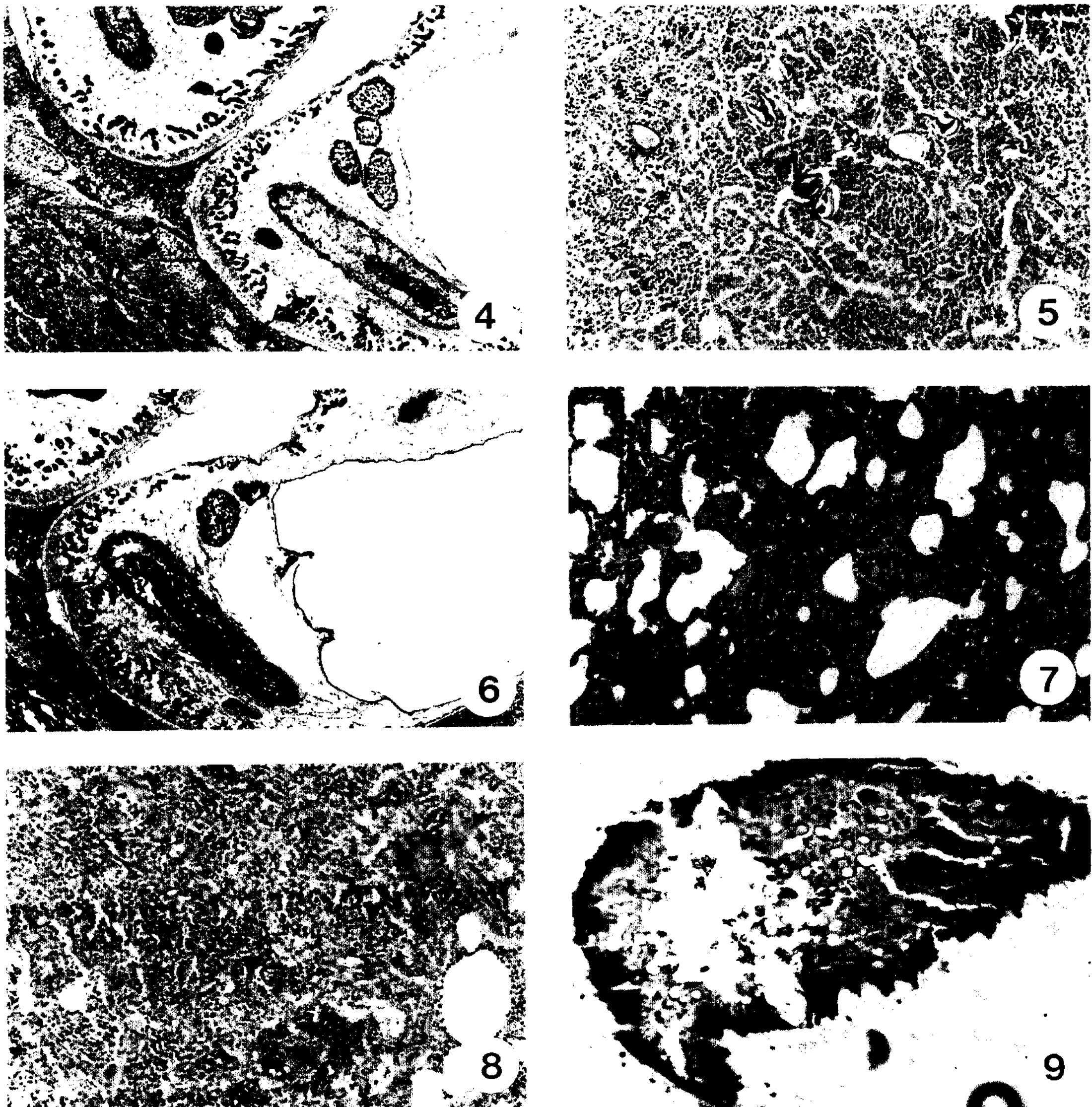
in infected cat lung. It exhibited brown colored intestinal content. Fig. 5 showed a granuloma wall section of the infected cat lung with interspersed, sectioned eggs of *Paragonimus.* Within yellow egg shell, hematoxylin-stained egg cells were recognized.

Figs. 6 and 7 revealed the binding localities of polyclonal antibody in an infected human serum. Strong reactions of AEC chromogen were recognized at intestinal epithelial border, intestinal content, border of excretory bladder and eggs either in uterus (Fig. 6) or in cat lung cyst wall (Fig. 7). Positive reactions were also observed in outer tegumental layer and subtegumental layer while ovary, vitellaria, parenchymal tissue and tegument were faintly stained or not.

Fig. 8 showed the reactivity of PFCK-136 MAb. When the AEC chromogen was colorized, eggs in cat lung granuloma wall showed the positive reactions. Locality of PFCK-44 MAb was shown in Fig. 9. Positive reaction was recognized only in the intestinal epithelial border of the worm.

DISCUSSION

By immunohistochemistry using MAb of PFCK-136 as a primary antibody, it was confirmed that the band 1 protein in disc-PAGE of crude extract of adult *P. westermani* was the egg protein (Kim *et al.*, 1986; Imai and Nawa, 1988). This protein has been known to have molecular mass of 440 kDa in non-denatured state (Imai and Nawa, 1988; Kong *et al.*, 1991). In this study, subunits of this band 1 protein were proved to be 23, 46, and 92 kDa polypeptides when observed by SDS-PAGE/immunoblot using PFCK-136 (Fig. 1) or 23 kDa when observed by reducing SDS-PAGE (Fig. 3). In previous observations of SDS-PAGE of the crude extract of adult *P. westermani* and immunoblot using patients sera, 23, 46 and 92 kDa proteins were repeatedly recognized as strongly reactive antigens (Kim *et al.*, 1988; Cho *et al.*, 1989). The perplexing relations between SDS-PAGE/immu-



Figs. 4~9. Immunohistochemical stainings of *P. westermani* and its surrounding granuloma with MAbs and paragonimiasis patient serum. **Fig. 4.** Negative control (×100). Normal human serum was reacted as a primary antibody. No organs were reacted with AEC chromogen. **Fig. 5.** Negative control reacted with normal human serum on granuloma wall with eggs(×100). **Fig. 6.** A patient serum of paragonimiasis was used as a primary antibody. Strong reactions were shown (see text). **Fig. 7.** Section of eggs in granuloma wall reacted with a patient serum (×100). **Fig. 8.** Reactivity of PFCK-136 MAb to eggs in granuloma wall (×100). **Fig. 9.** Reactivity of PFCK-44 MAb to intestinal wall of the worm (×200).

noblot findings and egg protein became clear by this study.

Another purified protein, band 4 in disc-PAGE of crude saline extract of adult *P. westermani* was found in secretory-excretory product (Imai and Nawa, 1988). Its molecular mass has been estimated to be 13 kDa by gel filtration (Imai and Nawa, 1988) and 17 kDa by Ferguson plot (Kong *et al.*, 1991). By reducing SDS-PAGE of the purified band 4 protein (Fig. 3) and SDS-PAGE/immunoblot using PFCK-44 MAb (Fig. 1), its molecular mass was repeatedly

shown as 17 kDa. This indicated that the band 4 protein was a monomer in non-denatured state. This protein was produced at intestinal epithelium of the adult *P. westermani* as shown by immunohistochemistry (Fig. 9). In this connection, it was interesting that superoxide dismutase (SOD) of *P. westermani* had the same molecular mass of 17 kDa when observed by SDS-PAGE. But this enzyme was a intracellular dimer protein of 34 kDa in non-denatured state (Chung *et al.*, 1991). These findings suggested that the 17 kDa band in SDS-PAGE of the crude extract of the worm was composed of at least 2 different proteins such as the band 4 protein in the disc-PAGE and SOD. This assumption should be proved in the future. Because the 17 kDa band in SDS-PAGE of the worm extract was considerably antigenic in human paragonimiasis (Kim *et al.*, 1988), correct understading of their functions seems important.

Similar studies were made by Sugiyama *et al.* (1987, 1988) to purify proteins in the crude extract of *P. westermani*. One of their adult worm protein, purified by MAb, A1-2, was composed of 12.5, 15.5, 17 and 35 kDa subunits which reacted immunohistochemically to parenchymal tissue. Another purified protein (by A4-1 MAb), composed of 26 and 28 kDa, was found both in adult and larval extracts and was reacting to intestinal epithelium. The latter protein of 26/28 kDa has been known as a strong antigen in diagnosing paragonimiasis (Sugiyama *et al.*, 1987, 1988). In addition to it, the band 4 protein in disc-PAGE of adult extract of *P. westermani* was reacting to intestinal epithelium as observed in this study. These results suggested that the intestinal secretion were also composed of several different proteins which stimulated the antibody formation in the infected hosts. Their biological activity and physicochemical properties deserve more studies in the future.

REFERENCES

Cho, S.Y., Hong, S.T., Rho, Y.H., Choi, S. and Han, Y.C. (1981) Application of micro-ELISA in serodiagnosis of human paragonimiasis. *Korean J. Parasit.*, **19**:151-156.

Cho, S.Y., Kim, S.I., Kang, S.Y., Kong, Y., Han, S.K., Shim, Y.S. and Han, Y.C. (1989) Antibody changes in paragonimiasis patients after praziquantel treatment as observed by ELISA and immunoblot. *Korean J. Parasit.*, **27**:15-21.

Choi, W.Y., Yoo, J.E., Nam, H.W. and Choi, H.R. (1986) Purification of antigenic proteins of *Paragonimus westermani* and their applicability to experimental paragonimiasis. *Korean J. Parasit.*, **24**:177-186. (in Korean)

Chung, Y.B., Song, C.Y., Lee, H.S, Kong, Y. and Cho, S.Y. (1991) Purification and characterization of Cu, Zn-Superoxide dismutase from adult *Paragonimus westermani*. *Korean J. Parasit.*, **29**(3): 259-266.

Davis, B.J. (1964) Disc-electrophoresis II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.*, **121**:404-427.

Huer, B., Kim, S.I., Kang, S.Y. and Cho, S.Y. (1985) Electrophoretic patterns of proteins from *Paragonimus westermani* in early developmental stages. *Korean J. Parasit.*, **23**:189-196.

Imai, J. and Nawa, Y. (1988) Immunochemical characterization of major antigenic components of *Paragonimus westermani* (Triploid type) adult worms. *Jpn. J. Parasitol.*, **37**:398-404.

Joo, K.H., Ahn, H., Chung, M.S. and Rim, H.J. (1989a) Demonstration of species-specific and cross-reactive components of *Paragonimus wester mani* crude worm antigen by EITB. *Korean J. Parasit.*, **27**:9-14.

Joo, K.H., Hong, S.C., Chung, M.S. and Rim, H.J. (1989b) Analysis of antigenic specificities of *Paragonimus westermani* developmental stages using immunoblot technique. *Korean J. Parasit.*, **27**:1-7. (in Korean)

Kim, S.H., Kong, Y., Kim, S.I., Kang, S.Y. and Cho, S.Y. (1988) Immunoblot observation of antigenic protein fractions in *Paragonimus westermani* reacting with human patients sera. *Korean J. Parasit.*, **26**:239-243.

Kim, S.I., Ko, E.K., Kang, S.Y. and Cho, S.Y. (1986) Antigenicity of the soluble egg antigen of *Paragonimus westermani*. *Korean J. Parasit.*, **24**: 49-54. (in Korean)

Kong, Y., Kim, W.B., Kang, S.Y. and Cho, S.Y. (1991) Molecular weight of major component

proteins in crude saline extract of *Paragonimus westermani*. *Korean J. Parasit.*, **29**(2):113-120.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*, **227**:681-685.

Lee, O.R. and Chang, J.K. (1986) ELISA of paragonimiasis in cat by crude and purified antigens of *Paragonimus westermani*. *Korean J. Parasit.*, **24**:187-193. (in Korean)

Lowry, O.H., Rosenbrough, N.J., Lewis-Farr, A. and Randall, R.J. (1951) Protein measurement with Folin-phenol reagent. *J. Biol. Chem.*, **193**: 265-275.

Pharmacia Fine Co. (1983) Affinity chromatography: Principles and methods. Pharmacia Fine Co., Ljungfoeretagen AB.

Shim, Y.S , Cho, S.Y. and Han, Y.C. (1991) Pulmonary paragonimiasis: A Korean perspective. *Seminars in Resp. Med.*, **12**:35-45.

Slemenda, S.B., Maddison, S.E., Jung, E.C. and Moore, D.D. (1988) Diagnosis of paragonimiasis by immunoblot. *Am. J. Trop. Med. Hyg.*, **39**: 469-471.

Sugiyama, H., Hinoue, H., Katahira, J., Horiuchi, T., Tomimura, T., Kamata, Y. and Kozaki, S. (1988) Production of monoclonal antibody to characterize the antigen of *Paragonimus westermani*. *Parasitol. Res.*, **75**:144-147.
mani antigen stimulating antibody formation in both infected cat and rat. *J. Parasitol.*, **73**:363-367.

Sugiyama, H., Sugimoto, M., Akasaka, K., Horiuchi, T., Tomimura, T. and Kozaki, S. (1987) Characterization and localization of *Paragonimus wester-*

Tsang, V.C.M., Peralta, J.M. and Simons, A.R. (1983) Enzyme-linked immunoelectrotransfer blot technique (EITB) for studying specificities of antigens and antibodies separated by gel electrophoresis. *Methods in Enzymol.*, **92**:377-391.

Yokogawa, M. (1982) Paragonimiasis. In: *CRC Handbook Series in Zoonoses* (*Vol. III*), p. 123, CRC Press, Boca Raton.

=국문요약=

친화성크로마토그래피로 순수분리한 폐흡충 성충 성분단백질의 성상

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인체감염이 다발하는 폐흡충증의 혈청학적 진단에서 항원으로 사용하는 성충추출액은 여러단계의 질환 이행과정을 진단하는 항원으로서 문제가 있다. 이를 해결하려면 먼저 추출액내의 성분단백질의 성상을 파악할 필요가 있다. 이 연구에서는 폐흡충 성충 추출액으로 면역시킨 BALB/c mice의 비장세포와 SP2/0 형질세포종 세포를 세포융합하여 제작한 단세포군항체를 이용하여 친화성크로마토그래피로 폐흡충 성충의 구성단백질의 일부를 순수분리하고 성상을 관찰하였다. 그 결과는 다음과 같다.

1. 세포융합으로 PFCK-21, PFCK-44, PFCK-136, PFCK-189 등 4종류의 단세포군 항체를 얻었다. 그중 PFCK-21과 PFCK-44는 17k Da, PFCK-136은 23, 46, 92 kDa 단백질에 반응하였고 PFCK-189는 여러종류의 단백질에 반응하였다.

2. PFCK-44 단세포군항체를 고리로 친화성 크로마토그래피를 실시하여 분리한 성분 단백질은 disc-PAGE상 4번째에 위치하고 분자량이 17 kDa로 알려진 단백질이었다. 이 단백질은 17 kDa의 monomer로 판단하였다. 면역조직화학염색을 실시한 결과 이 단백질은 장관 상피세포에 반응하고 있었다.

3. PFCK-136 단세포군항체로 순수분리한 단백질은 disc-PAGE상 1번 단백질(440 kDa)이었으며 환원성 SDS-PAGE에서는 23 kDa 단백질이었다. 면역조직화학염색으로 이 단세포군항체는 충란내 세포에 강하게 반응하고 있었다.

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