COMPARATIVE STUDIES ON THE ANTIGENIC STRUCTURES OF FIVE SUBSPECIES OF *ONCOMELANIA* SNAILS BY IMMUNOELECTROPHORESIS

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Abstract. To approach the biochemical relationships of five subspecies of *Oncomelania* snails, antigenic structures among the subspecies were compared using immunoelectrophoresis. The results obtained are summarized as follows:

1) For five subspecies of *Oncomelania hupensis* snails (*Oncomelania hupensis hupensis, O.h.nosophora, O.h. formosana, O.h. chiui* and *O.h. quadrasi*), 23-24 precipitin bands were observed between the antigens and their homologous antisera, while 18-22 bands were observed in the heterologous reactions.

2) For each subspecies, residual bands observed after absorption procedure demonstrated the presence of antigens unique to each subspecies except *O.h. chiui.*

Based on the immunological antigenic structures among the *Oncomelania* subspecies, it is suggested that *O.h. nosophora* and *O.h. hupensis* forms are closely related group, while *O.h. formosana, O.h. chiui* and *O.h, quadrasi* forms are another group.

INTRODUCTION

It is well-known that there are five recognized subspecies of *Oncomelania* snails. The subspecies have distributed to the Japan, China, Taiwan and Philippines. Recently, in addition to these five subspecies: *Oncomelania hupensis nosophora, O.h. hupensis, O.h. formosana, O.h. chiui* and *O.h. quadrasi*, Davis *et al* (1988) added *O.h. lindoensis*, restricted to the island of Sulawesi. However, they reported that *O.h. lindoensis* was presumably derived secondarily from Philippines snails.

Oncomelania snails serve as intermediate hosts of the Asian blood fluke, *Schistosoma japonicum*. Many investigators reported that the different geographical strains of *S. japonicum* showed different infectivities to various subspecies of *Oncomelania* snails (Moose and Williams, 1963; Lee *et al*, 1982; Iwanaga, 1976). Moose and Williams (1964) reported that *O.h. formosana* was refractory to infection with the human strain of *S. japonicum*, even though *O.h. formosana* was taxonomically related to the other four subspecies of *Oncomelania* snails (Davis, 1967). This finding suggests the existence of basic physiologic and biochemical differences among *Oncomelania* snails.

The present paper deals with antigenic structures among five subspecies of *Oncomelania* snails by immuoelectrophoresis.

MATERIALS AND METHODS

Source of *Oncomelania* snails

Five subspecies of laboratory-reared *Oncomelania* snails were employed in the experiments. The laboratory colonies were originated from the following areas; *Oncomelania hupensis nosophora* from Yamanashi, Japan; *O.h. hupensis* from Shanghai, China; *O.h. formosana* from Changhua, Taiwan; *O.h. chiui* from Shihmen, Taiwan; *O.h. quadrasi* from Leyte, Philippines.

Oncomelania snails were reared in the laboratory following the cultivation techniques of Iwanaga (1973, 1975).

Preparation of antigens and antisera

Oncomelania snails antigens were prepared from 0.1% NaCl extracts of the whole body according to

the methods of Tsuji (1974) and Iwanaga and Tsuji (1985). Antisera (*ie*, immune sera) to *Oncomelania* snails were prepared by the following method: Emulsion containing 2-3 mg/ml of each *Oncomelania* snail antigen in 1.0-1.5 ml of Freund's complete adjuvant (DIFCO Lab, Detroit. USA) were injected into proximal limbs of rabbits intramuscularly for ten times every week, and antisera were obtained from blood drawn from these rabbits ten days after the final injection (modification of Tsuji, 1974, 1975).

Absorption procedure

0.4-0.6 ml of the antiserum to a specific antigen was adsorbed with 8mg of another antigen. After stirring, the antiserum-antigen mixture was incubated for 3 hours at 37°C and then stored 12 hours at 4°C. The mixture was centrifuged at 3,000 rpm for 5 minutes. After centrifugation, the supernatant was used as absorption antiserum (modification of Tsuji, 1974).

Immunoelectrophoresis

Immunoelectrophoresis was done according to the technique of Tsuji (1974, 1975) on 0.9% agarose gel (Behringwerke, AG, Germany) in veronal buffered saline (pH 8.2) and the electric current, adjusted to 18 ± 2V/8cm gel length, was applied for 3 hours.

RESULTS

In the homologous antigen-antibody systerms, 24 precipitin bands were observed with each of *O. h. nosophora, O. h. hupensis* and *O. h. formosana*, similarly, 23 bands were observed with each of *O. h. chiui* and *O. h. quadrasi* as shown in Table 1 and Fig 1. Heterologous reactions among *Oncomelania* snails produced fewer precipitin bands than did homologous reactions (Table 1). The antiserum from rabbits immunized with *O. h. nosophora* showed 21 bands with *O. h. hupensis* antigen, 18 bands with each of *O. h. formosana* and *O. h. chiui* antigens and 19 bands with *O. h. quadrasi* antigen. In cases of anti-*O. h. hepensis* serum, it was possible to demonstrate 21 bands with *O. h. nosophora* antigen, 18 bands with each of *O. h. formosana* and

Table 1

Number of precipitin bands among *Oncomelania* snails.

Antigens	Immune sera				
	OHN	OHH	OHF	OHC	OHQ
OHN	24	21	18	18	19
OHH	21	24	18	18	19
OHF	18	18	24	22	20
OHC	18	18	22	23	20
OHQ	19	19	20	20	23

OHN: *O. h. nosophora* OHH: *O. h. hupensis*
OHF: *O. h. formosana* OHC: *O. h. chiui*
OHQ: *O. h. quadrasi*

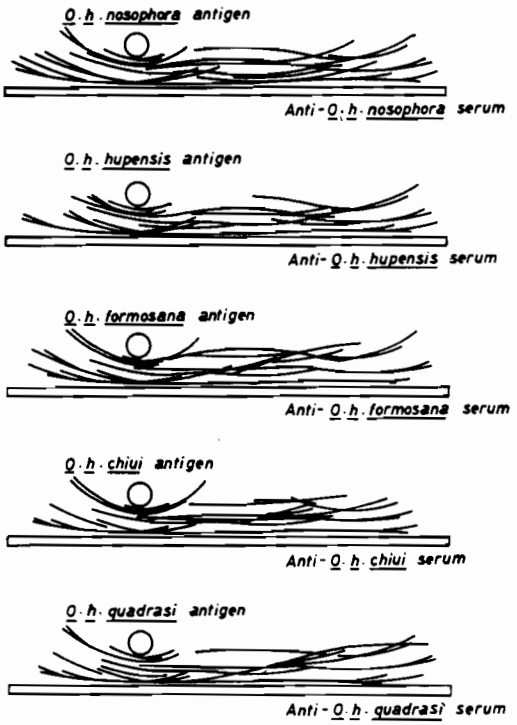

Fig 1–Immunoelectrophoregrams between the antigens and their homologous antisera of *Oncomelania* snails.

Table 2

Number of residual bands after absorption.

Antigen used for test	Immune sera absorbed				
	OHN	OHH	OHF	OHC	OHQ
OHN	0	3	6	5	4
OHH	3	0	6	5	4
OHF	6	6	0	1	3
OHC	6	6	2	0	3
OHQ	5	5	4	3	0

OHN: *O. h. nosophora* OHH: *O. h. hupensis*
OHF: *O. h. formosana* OHC: *O. h. chiui*
OHQ: *O. h. quadrasi*

O. h. chiui antigens and 19 bands with *O. h. quadrasi* antigen. The anti-*O. h. formosana* serum showed 18 bands with each of *O. h. nosophora* and *O. h. hupensis* antigens, 22 bands with *O. h. chiui* antigen and 20 bands with *O. h. quadrasi* antigen. And the anti-*O. h. chiui* serum showed 18 bands with each of *O. h. nosophora* and *O. h. hupensis* antigens, 22 bands with *O. h. formosana* antigen and 20 bands with *O. h. quadrasi* antigen. Finally, the anti-*O. h. quadrasi* serum showed 19 bands with each of *O. h. nosophora* and *O. h. hupensis* antigens, and 20 bands with each of *O. h. formosana* and *O. h. chiui* antigens.

Absorption procedures were performed to compared antigenicities among *Oncomelania* snails (Table 2). Three, six, six and five bands respectively were recognized as the residual reaction of anti-*O. h. nosophora* serum absorbed with each of *O. h. hupensis, O. h. formosana, O. h. chiui* and *O. h. quadrasi* antigens, respectively (Figs 2, 3). Using anti-*O.h. hupensis* serum, three, six, six and five bands respectively were recognized as the residual bands after absorptions with each of *O. h. nosophora, O. h. formosana, O. h. chiui* and *O. h. quadrasi* antigens, respectively (Fig 4). Anti-*O. h. formosana* serum absorbed with each of *O. h. nosophora* and *O. h. hupensis* antigens produced six bands in each case, similarly, two bands with *O. h. chiui* antigen and four bands with *O. h. quadrasi* antigen (Fig 5). The immunoelectrophoregram in Fig 6 showed residual bands of anti-*O. h. chiui* serum absorbed with each of *O. h. nosophora, O. h. hupensis, O. h. formosana* and *O. h. quadrasi* antigens; 5 bands each were recognized with *O. h. nosophora* and *O. h. hupensis* antigens, 1 band with *O. h. formosana* antigen and 3 bands with *O. h. quadrasi* antigen. In

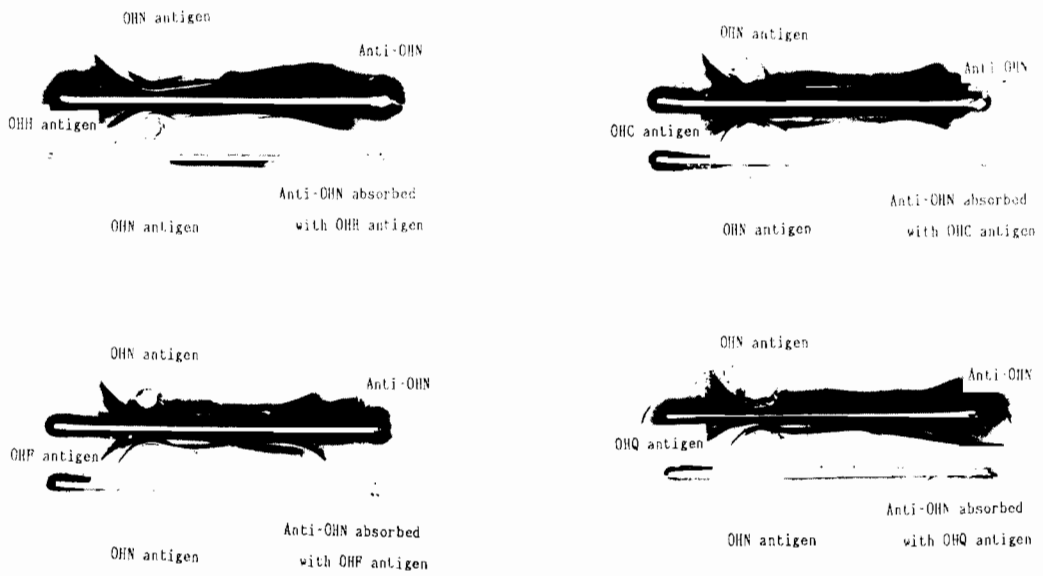

Fig 2–Immunoelectrophoresis between *Oncomelania hupensis nosophora* and other *Oncomelania* snails using absorption procedures. OHN: *O. h. nosophora* OHH: *O. h. hupensis* OHF: *O. h. formosana* OHC: *O. h. chiui* OHQ: *O. h. quadrasi*

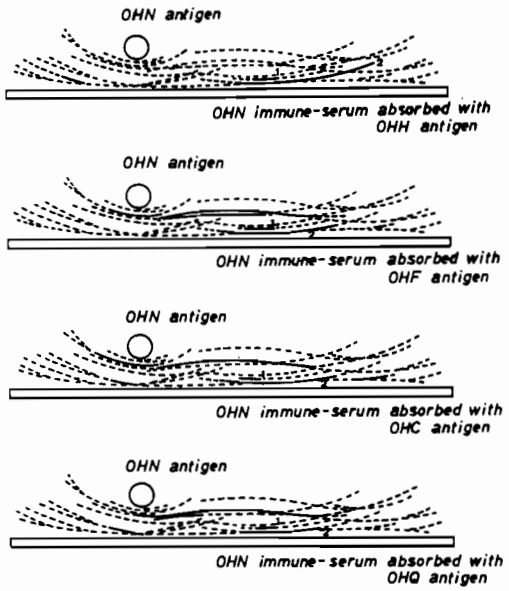

Fig 3–Immunoelectrophoregrams between *Oncomelania hupensis nosophora* and other *Oncomelania* snails using absorption procedures.
Dotted lines: Common bands with both antigens
Bands No. 1 and No. 2: Specific bands for *O. h. nosophora*
OHN: *O. h. nosophora* OHH: *O. h. hupensis*
OHC: *O. h. chiui* OHF: *O. h. formosana*
OHQ: *O. h. quadrasi*

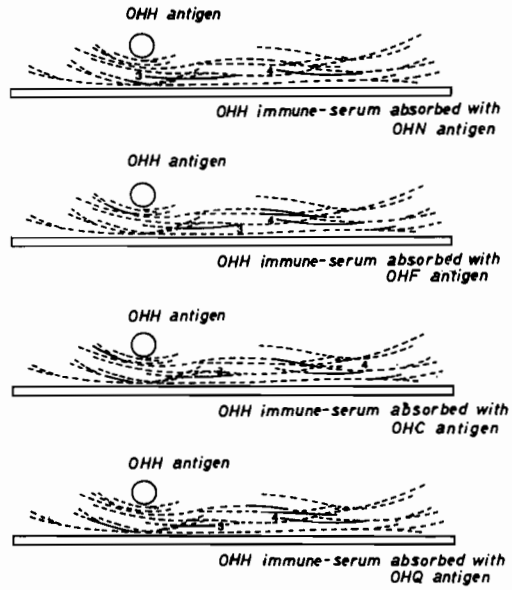

Fig 4–Immunoelectrophoregrams between *Oncomelania hupensis hupensis* and other *Oncomelania* snails using absorption procedures.
Dotted lines: Common bands with both antigens
Bands No. 3 and No. 4: Specific bands for *O. h. hupensis*
OHH: *O. h. hupensis* OHN: *O. h. nosophora*
OHC: *O. h. chiui* OHF: *O. h. formosana*
OHQ: *O. h. quadrasi*

the anti-*O. h. quadrasi* serum (Fig 7), 4 bands were recognized as the residual bands after absorption with each of *O. h. nosophora* and *O. h. hupensis* antigens, and 3 bands were noticed after absorption with each of *O. h. formosana* and *O. h. chiui* antigens. From the results of absorption among *Oncomelania* snails, common residual unreacted bands remained for each specific heterologous antigen-antiserum reaction, suggested the presence of one or more antigens unique to each subspecies. Thus, bands No. 1 and No. 2 were common residual bands (*ie* specific bands) for *O. h. nosophora* absorbed with each of four other subspecies of *Oncomelania* snails. Similarly, common residual bands for *O. h. hupensis* were bands No. 3 and No. 4, No. 5 for *O. h. formosana*, and No. 6 for *O. h. quadrasi*

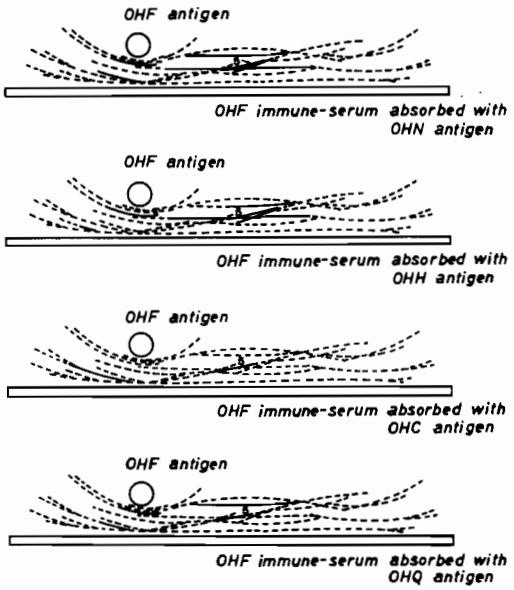

Fig 5–Immunoelectrophoregrams between *Oncomelania hupensis formosana* and other *Oncomelania* snails using absorption procedures.
Dotted lines: Common bands with both antigens
Band No. 5: Specific band for *O. h. formosana*
OHF: *O. h. formosana* OHN: *O. h. nosophora*
OHH: *O. h. hupensis* OHC: *O. h. chiui*
OHQ: *O. h. quadrasi*

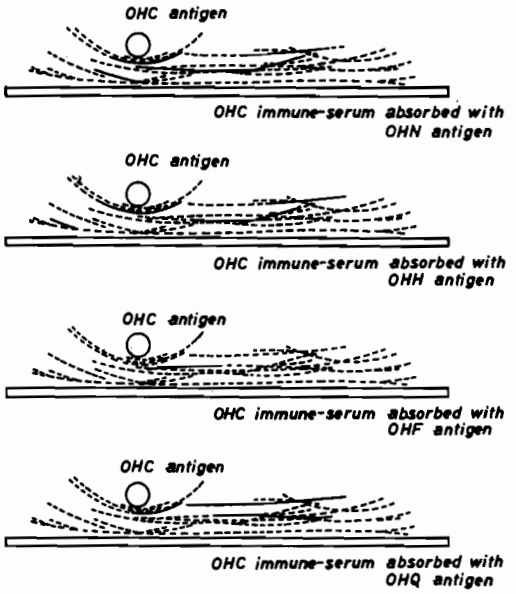

Fig 6–Immunoelectrophoregrams between *Oncomelania hupensis chiui* and other *Oncomelania* snails using absorption procedures.
Dotted lines: Common bands with both antigens
OHC: *O. h. chiui* OHN: *O. h. nosophora*
OHH: *O. h. hupensis* OHF: *O. h. formosana*
OHQ: *O. h. quadrasi*

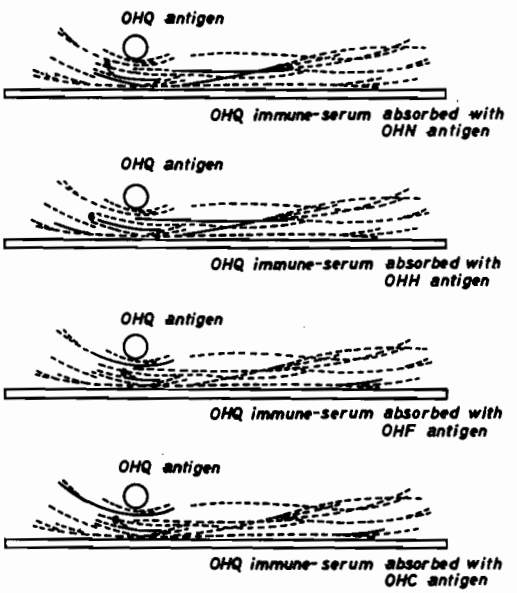

Fig 7–Immunoelectrophoregrams between *Oncomelania hupensis quadrasi* and other *Oncomelania* snails using absorption procedures.
Dotted lines: Common bands with both antigens
Band No. 6: Specific band for *O. h. quadrasi*
OHQ: *O. h. quadrasi* OHN: *O. h. nosophora*
OHH: *O. h. hupensis* OHF: *O. h. formosana*
OHC: *O. h. chiui*

(Figs 3, 4, 5, 7). No common residual band, specific to *O. h. chiui*, was observed (Fig 6).

DISCUSSION

Immunoelectrophoresis has been extensively utilized for immunological aspects of physiological studies of parasites and snails (Biguet *et al*, 1962; Santana *et al*, 1992; Iwanaga *et al*, 1992). Species discrimination of snails was accomplished by immunological methods (Tran Vay Ky *et al*, 1962; Rosé *et al*, 1966; Davis, 1969). Iwanaga (1992) reported immunological differences of Brazilian species of *Biomphalaria* snails by immunoelectrophoresis, and suggested that *Biomphalaria glabrata* pigmentado, *B. glabrata* albino and *B. straminea* were not same species, although *B. glabrata* pigmentado and *B. glabrata* albino were closely related to each other.

In the present study (Table 1), it was found that *O. h. nosophora* shared between 18 and 21 antigens with each of the other four subspecies of *Oncomelania* snails, and shared the largest number of bands (21) with *O. h. hupensis*. Thus, *O. h. nosophora* and *O. h. hupensis* are most closely related to each other immunoelectrophoretically. Furthermore, *O. h. nosophora* produced the fewest common bands (18) with *O. h. formosana* and *O. h. chiui*. Comparing immunoelectrophoretical patterns between *O. h. formosana* and *O. h. chiui*, these two subspecies were most closely related to each other (22 shared bands), similarly, *O. h. quadrasi* was more closely related to *O. h. chiui* and *O. h. formosana* than to either *O. h. nosophora* or *O. h. hupensis*. These findings are in agreement with the report by Davis and Lindsay (1967) on disk electrophoretic profiles of proteins of *O. h. nosophora*, *O. h. chiui* and *O. h. formosana*.

Absorption procedures were performed to compare antigenic specificities among *Oncomelania* snails. Some residual bands among *Oncomelania* snails showed different patterns, suggesting that each *Oncomelania* subspecies had different antigenicities.

Recently, enzyme biochemistry of *Oncomelania* snails were studied (Viyanant *et al*, 1987; Woodruff *et al*, 1988). Tsukamoto *et al* (1988) reported the isozyme patterns among *O. h. nosophora, O. h. formosana* and *O. h. quadrasi* by discontinuous electrophoresis in 5% polyacrylamide gels. For the 16 enzymes examined, some showed differences in electrophoretic mobility of banding patterns in the three subspecies. However, they suggested that *O. h. nosophora, O. h. formosana* and *O. h. quadrasi* were closely related to each other as a species complex.

In this study, some common antigens were found in the comparison of heterologous reactions. Furthermore, from the results of absorption procedures, the five subspecies of *Oncomelania* showed some antigenic differences. For each subspecies, a common residual band present after absorption against each other subspecies, and demonstrates the presence of antigens unique to each subspecies. In particular, residual bands No. 1 to No. 6 are specific bands for each *Oncomelania* subspecies, but these residual bands are very weak. Further, biochemical analysis of these residual bands, using SDS-PAGE and/or Western blotting techniques, will be necessary to confirm these differences.

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