

Seasonal Effects on Clonorchicidal Substances from Epidermal Mucus of *Cyprinus carpio*, *Ophicephalus argus* and *Parasilurus asotus*

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Some fresh-water fishes are responsible for the second intermediate host of *Clonorchis sinensis* and others are not although they all inhabit the same water system. As a series of studies to clarify this mechanism, clonorchicidal substances were fractionated from the body surface mucus of *Carassius carassius*, *Cyprinus carpio*, *Parasilurus asotus*, *Ophicephalus argus*, *Cyprinus carpio nudus*, etc, by means of silica gel thin layer and column chromatography (Rhee *et al.*, 1979, 1980a & 1983). Rhee *et al.* (1984) have recently reported that the substance from the mucus of *C. carpio* belong to an ethyl ester of unsaturated fatty acid with 2 double bonds, 15 methylene groups and 1 methyl group. Rhee *et al.* (1980b) also observed that in the ether extract from the mucus of *C. carassius*, not only the *Rf* values of the uppermost first spot by thin layer chromatography but likewise the clonorchicidal substance has seasonal variation and that the wormicidal activity of the substance is the strongest in summer. In *C. carpio nudus*, however, no seasonal variation was found in the *Rf* values and the effects of wormicidal fractions (Rhee *et al.*, 1983).

In the present study, seasonal variations of the substances of *C. carpio*, *O. argus* and *P. asotus* were observed. At intervals of 2 months from August 1981 to June 1982, each time 1kg of the fishes was collected for 3 species, and the fishes were exfoliated to obtain epidermis and dermis from the bodies. They were extracted by soaking in ether for 24 hours and the

extracts were concentrated by rotary vacuum evaporation method and stored at 5°C.

Each extract was diluted 100 times with ether, and then 1ml of the diluent was transferred into a convex dish and the solvent was evaporated. After adding into the dish, 0.5ml of tap water which contained more than 20 fresh *Clonorchis* cercariae or 0.5ml of normal saline solution which included more than 30 fresh excysted metacercariae of *Clonorchis*, the viability of the cercariae and the metacercariae was observed *in vitro* under stereomicroscopy. Two ml normal saline solution or tap water was added to each experimental plot when the larvae are at near death and dead cercariae/metacercariae were discriminated from living ones by their motility.

As shown in Table 1, the amounts of the ether extracts from the mucus of 3 species of fishes in April, June, August and October were greater than those in February and December. From the results obtained by bi-monthly survey for wormicidal activity in epidermal mucus from 3 species of fishes as shown in Table 2, present authors could recognize almost no seasonal variation in clonorchicidal activity of the mucus from 3 species of fishes in spite of the quantitative increase of the ether extracts from April to October. Wormicidal activity of *C. carpio* was, in general, stronger as compared with those of *O. argus* and *P. asotus*. In the control, the cercariae and the metacercariae continued to survive over 480 and 600 minutes, respectively.

Table 1. Amount* of ether extracts from body surface mucus of *Cyprinus carpio*, *Ophicephalus argus* and *Parasilurus asotus* (Unit: g)

Fishes	Months					
	February	April	June	August	October	December
<i>Cyprinus carpio</i>	13.4	20.1	19.1	18.0	18.8	10.5
<i>Ophicephalus argus</i>	13.8	17.8	18.3	17.5	16.5	9.8
<i>Parasilurus asotus</i>	9.8	18.7	15.5	16.8	13.0	7.2

*These figures are obtained from each 1kg fish.

Table 2. Time* required for killing *Clonorchis* larvae in ether extracts from body surface mucus of *Cyprinus carpio*, *Ophicephalus argus* and *Parasilurus asotus* (Unit: Minutes)

Fishes	<i>Cyprinus carpio</i>		<i>Ophicephalus argus</i>		<i>Parasilurus asotus</i>	
	Cercaria	Metacercaria	Cercaria	Metacercaria	Cercaria	Metacercaria
February	81.0±9.4	194.0±31.9	176.7±8.5	211.0±21.4	148.0±10.7	216.3±14.9
April	77.3±13.2	168.3±20.5	169.7±20.2	189.3±10.6	138.0±16.5	189.7±28.6
June	75.7±10.8	160.7±29.2	130.3±20.4	197.7±14.0	124.7±32.3	171.3±17.7
August	109.3±9.4	196.0±19.0	112.7±11.0	202.3±8.8	94.0±20.0	200.7±11.7
October	112.7±7.1	193.7±15.1	145.7±25.8	208.3±27.1	109.3±10.5	209.3±11.4
December	79.0±8.8	156.3±17.4	153.7±14.6	203.7±25.9	119.3±12.0	213.0±15.6

*In the controls, the cercariae and the metacercariae survived over 480 and 600 minutes, respectively. Each value represents the mean of triplicate determinations with the standard deviations.

Meanwhile, the ether extracts were fractionated into 5 spots in *C. carpio* and *O. argus* and 3 spots in *P. asotus* on silica gel thin layer chromatography with petroleum ether/chloroform(30/70, v/v). Almost no seasonal variation in the *Rf* values of various spots was also realized.

Consequently, previous work (Rhee *et al.*, 1980b & 1983) and this experimental results suggest that seasonal variation in clonorchicidal substance from the mucus is merely due to a difference among species of fishes used.

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== 국문초록 ==

잉어, 가물치 및 메기의 體表粘液에 있는 肝吸蟲 殺蟲性物質의 季節的 變動

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잉어, 가물치 및 메기의 體表面 粘液內에 있는 肝吸蟲에 대한 殺蟲性物質의 季節的 變動에 대하여 *in vitro*에서 檢討하였다.

이들 魚類 體表粘液의 에테르抽出物量은 겨울철에는 그 밖에 季節에 비하여 현저하게 감소함에도 불구하고 殺蟲性物質과 에테르抽出物을 薄膜 크로마토그라피하여 얻은 各斑點物質의 *Rf*값은 季節的 變動을 가지오지 않았다.