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Research Note

Electrophoretic Studies on Enzymes in Japanese Common Liver Fluke, *Fasciola* sp. II. On Further Three Enzymes, GPI, GDH and ME, in the Liver Fluke

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Electrophoresis is a widely accepted technique for identification and/or classification of the species groups (Markert, 1975; Nozawa *et al.*, 1977). In the parasitic species, many studies on electrophoresis of enzymes have been reported (Carter, 1978; Ross *et al.*, 1978; Agatsuma and Suzuki, 1981). However, there is little information on isozymes in the Japanese common liver fluke, *Fasciola* sp.

Recently, Agatsuma and Suzuki (1980) performed electrophoretic investigations on four enzymes in the liver fluke. And they emphasized the finding of the considerably low variations of the enzymes in the liver fluke sampled from the natural population. The present paper reports three enzymes, glucosephosphate isomerase (GPI), NAD-dependent glutamate dehydrogenase (GDH) and NADP-dependent malic enzyme (ME) in the liver fluke, in addition to the four enzymes described previously (Agatsuma and Suzuki, 1980).

The adults of *Fasciola* sp. were obtained from the cattle bile ducts at the slaughter house in Kochi in 1979–1980. Totally, the sixty-one individuals of the liver fluke were examined for each enzyme. The samples for electrophoresis, including the host, cattle liver, were prepared in the same way as reported previously (Agatsuma and Suzuki, 1980). Electrophoretic techniques were also carried out essentially according to the methods of Agatsuma and Suzuki (1981). The buffer systems and conditions of electrophoresis for the three enzymes are given in Table 1. The incubation solutions for each enzyme are listed in Table 2. The preparation of reaction mixture is the same as described previously (Agatsuma and Suzuki, 1981).

As shown in Fig. 1, the electrophoretic patterns of all of the three enzymes were different between parasite and host. Although a source of failure to detect ME activity of the host was unknown, the result obtained was highly reproducible.

Six bands of GPI were detected and all migrated anodally. All the individuals examined possessed the same band patterns of GPI, showing no enzyme variation in the natural population examined. GDH showed a anodally-migrating single band, but tended to diffuse, demonstrating no variation. On the other hand, ME of all individuals migrated cathodally as a single

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Table 1 Buffer systems and condition of electrophoresis used in starch gel electrophoresis

Enzyme	Gel buffer	Electrode buffer	Condition
GPI, GDH ME	Dilute 100 ml of electrode buffer to 1000 ml	0.1 M Tris 0.1 M Maleic acid 0.01 M Na_2EDTA 0.01 M $MgCl_2$ 0.13 M NaOH	70 mA current constant, 16 h

Table 2 Enzyme assay systems for specific staining on electrophoresis

Enzyme	Substrate	Coenzyme	Other additions	Buffer conditon
GPI	Fructose-6-phosphate 3.0 mg	NADP 2.0 mg	MTT 2.0 mg PMS 2.0 mg $MgCl_2$ 74 mg G6PDH 2 U	0.1 M Tris-HCl (pH 8.0) 20 ml
GDH	Glutamate 100 mg	NAD 2.0 mg	MTT 2.0 mg PMS 2.0 mg	〃
ME	Malate 20 mg	NADP 2.0 mg	MTT 2.0 mg PMS 2.0 mg $MgCl_2$ 74 mg	〃

NAD, nicotinamide adenine dinucleotide. NADP, nicotinamide adenine dinucleotide phosphate. MTT, 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazolium bromide. PMS, phenazine methosulfate. G6PDH, glucose-6-phosphate dehydrogenase.

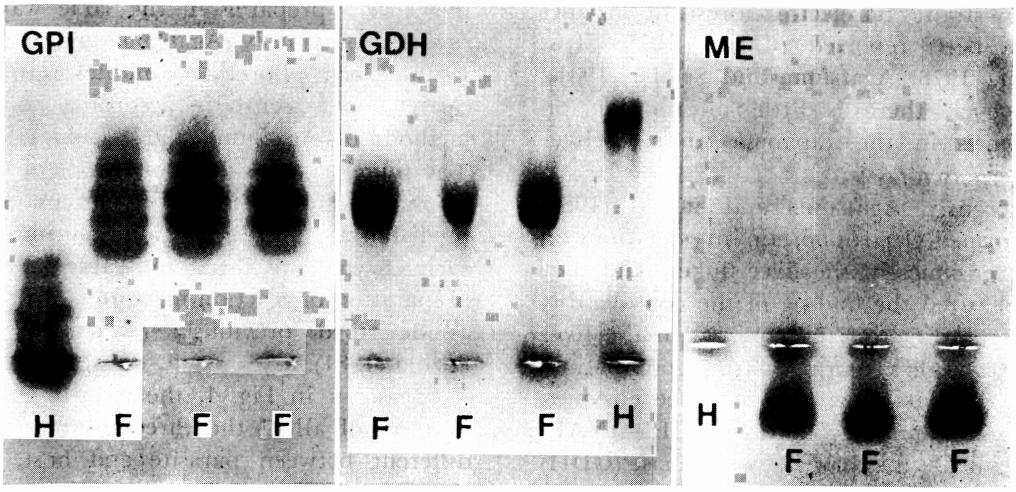

Fig. 1 Photograph of starch gel electrophoretic patterns of three enzymes in the Japanese common liver fluke, *Fasciola* sp. F, *Fasciola* sp.; H, host, cattle liver.

band, but a broad a little, showing the same mobilities; no variation was found. On the whole, it was found that there existed no variation of each enzyme in the liver fluke population examined in the present study.

Little variation has been already recognized in the previous investigation on the four enzymes of the Japanese common liver fluke sampled from the natural population (Agatsuma and Suzuki, 1980). The present study on the three enzymes, GPI, GDH and ME, confirmed again that the local population of the Japanese liver fluke showed markedly reduced enzyme variation. This phenomenon might be expected to occur due to their reproductive method, probably

parthenogenesis, as discussed in the previous report (Agatsuma and Suzuki, 1980). In order to know overall genetic diversity of the Japanese common liver fluke population, therefore, further survey of other populations will be necessary regarding these enzymes.

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Summary

Three enzymes, glucosephosphate isomerase (GPI), NAD-dependent glutamate dehydrogenase (GDH) and NADP-dependent malic enzyme (ME) were examined by means of starch gel electrophoresis, in addition to the previously reported enzymes. All of these three enzymes showed no variation in natural population. Thus, it was found again that the local population of the liver fluke showed markedly reduced enzyme variation.

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短　報

日本産肝蛭（*Fasciola* sp.）酵素の電気泳動法による研究
II. 酵素3種類，GPI，GDH，MEについて

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日本産肝蛭（*Fasciola* sp.）は，分類学的に多くの問題を含んでおり，その分類学的位置は，まだ明らかでない．本研究では，日本産肝蛭の分類学的研究の一つの試みとして，電気泳動法を用いて，その酵素の泳動パターンを調べた．今回は，すでに報告した4種類の酵素に加えて（Agatsuma and Suzuki, 1980），さらに3種類の酵素，glucosephosphate isomerase (GPI)，malic enzyme (ME) をデンプンゲル電気泳動法を用いて，その泳動パターンを検出し，自然集団における変異の割合も検討した．その結果，これらの酵素は，3種類とも，自然地方集団（61個体）では，変異は見られず，どの個体もそれぞれの酵素で同一のパターンを示した．この結果は，以前の報告（Agatsuma and Suzuki, 1980）と一致し，日本産肝蛭の地方集団では，酵素の変異が極めて少いことが，再び明らかとなった．