Electrophoretic Analyses of the Crop Contents of *Helobdella stagnalis* (L.) (Hirudinea)

Albert Keim

Limnologisches Institut der Universität Freiburg, Konstanz-Egg

(Z. Naturforsch. **32 c**, 739–742 [1977]; received April 1/July 11, 1977)

Electrophoretic Pattern, Crop Contents, Identification of Prey Species

First, experiments were tested for a new method concerning analyses of crop contents in liquosomatophagous animals. Disc-electrophoretic separations of the crop contents of *Helobdella stagnalis* yielded protein patterns typical for the number of bands and for the relative mobilities in these Chironomid larvae, on which *H. stagnalis* were fed. Additionally, slowly migrating bands occurred in the separations of the crop contents, varying in their number; likewise, an esterase showed a high activity at a crop contents of pH 6.2.

Introduction

Analyses of crop contents in animals providing their food intake in the liquid mode (liquosomatophages) are of interest for several reasons:

1. the host specifity of temporary blood suckers may indicate the epidemiology of diseases transferred by them;
2. food is an important factor in the ecologic niche for many animals, and
3. in certain ecosystems, some liquosomatophagous secondary consumers are important links in food chains.

Hitherto, serological methods have been applied to identify host and prey animals of liquosomatophages. A reliable identification of host and prey is fundamentally possible [1] yet fails in practice, due to the material required for the production of antiserum being not always available in sufficient amounts, as is particularly the case with rare and small species.

The possibility of a species identification of crop contents by applying other biochemical methods should therefore be examined. The protein electrophoresis appeared to be an appropriate method. The discontinuous polyacrylamide gel electrophoresis produced the best distinguishable electrophoresis patterns hitherto obtained [2]. With taxonomic investigations this method makes it possible to assign quite definite proteins to known genera and species. Subspecies generally show homologous patterns, some of them differing partly in single protein bands [3, 4]. This method also allows the identification of hybrids [5–7]. Electrophoresis has so far not been applied to the identification of crop contents. This method would first require the preparation of an atlas showing the protein patterns of host or prey animals. The food spectrum of the liquosomatophages concerned could then be ascertained by comparing the separations of the crop contents with known protein patterns. For a first approach examinations should be made whether or not the protein patterns of prey and host animals, respectively, can be identified in the crop contents of predators and parasites, respectively.

Materials and Methods

Discontinuous polyacrylamide gel electrophoresis was employed as elaborated in Maurer's gel system No. 1 [3]. The leech *Helobdella stagnalis* was proved to be a suitable experimental feeding on various invertebrates and, for example, occurs in the Daphnia culture pool of the Constance Institute, as well as in the littoral zone of Lake Constance. When feeding, it first seizes the prey with its oral sucker; the proboscis thereupon penetrates the cuticula, and presumably releases tissue-lyzing substances into the interior of the prey. Together with the hemolymph the lysate is sucked in and stored in the crop caeca. If, for instance, the leech feeds on Chironomid larvae, the red color of the Chironomid hemolymph (hemoglobin) in the *Helobdella* crop is preserved and will not disappear before the crop contents pass into the intestine. Larvae of *Chironomus tentans* from the Daphnia culture pool, *Ch. plumosus* from Lake Constance, at a depth of 20 m, and *Ch. thummi* from zoo shops were employed as food animals. The crop contents of the leech fed one hour before was prepared on a slide, and rinsed with 1/10 mol Tris/HCl buffer (pH 8.3, weighted with 40% sucrose) into a homogenizing vessel. Subsequent to homogenization the mixture was centrifuged, and the exceeding top layer resulting therefrom was used in the electrophoresis. The gels were prepared according tho Davis' method [2] by which first the separating, then the concentrating, gels are

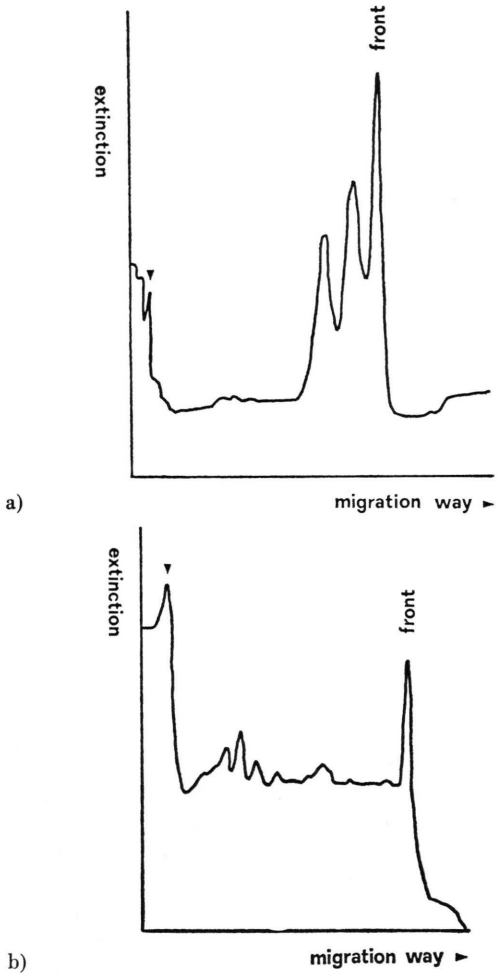

Fig. 1. Electropherogram of *Chironomus tentans* homogenate. a) Amido Black colouring. The arrow marks the beginning of the separating gel. The fast-migrating bands appear also in hemolymph pherograms. b) Esterases isozymes, measured at pH 7.1. The arrow mark the beginning of the separating gel.

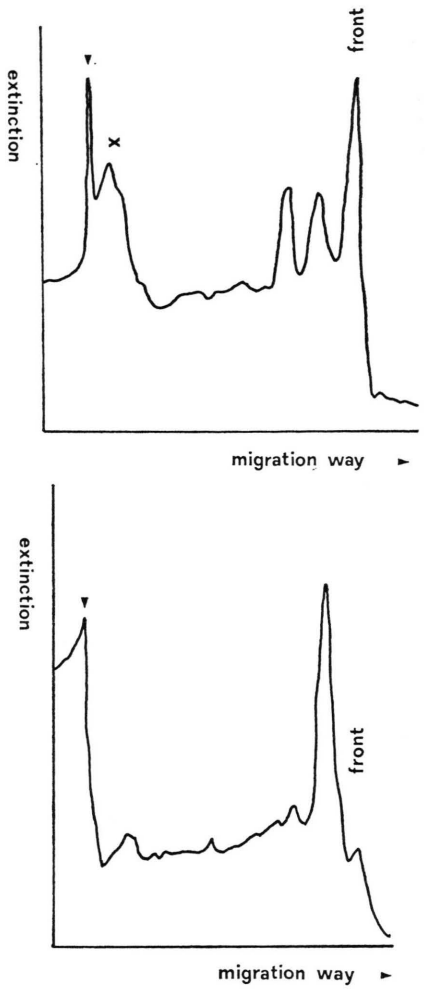

Fig. 2. Electropherogram of a crop content of *Helobdella stagnalis*. The animal has been fed on *Chironomus tentans*. a) Amido Black colouring. × marks crop content-specific slowly migrating bands. The other bands have the same R_F values as the bands in homogenate pherograms of *Chironomus tentans*. b) Esterase's measurement at the pH of the crop content of *H. stagnalis* (pH 6.2). Esterases isozymes of *Ch. tentans* are only provable in the front band. The great peak refer to an uniform intensive esterase activity. *Chironomus* proteins covered this crop content specific esterase at the examination of the whole protein with Amido Black. The arrow marks the beginning of the separating gel.

polymerized. 40 to 80 μg each of protein substance were poured into eight separate tubes. With each run a homogenate of *Ch. tentans* larvae was used for a standard. The separation was performed at 6 mA and 60 V in each tube (each having an inner diameter of 6 mm) during 30 min in a cooling chamber at +5 °C. After the gels had been removed from the tubes they were cut into two halves each along their longitudinal axis, and subjected to two different examinations:

1. determination of the whole protein by means of Amido Black 10 B [3];
2. determination of the unspecific esterases (100 mg Fast blue RR, 10 ml 0.5 mol citric acid buffer, 3 ml 1% α-naphtyl-acetate solution in acetone/water (1 : 1), 87 ml Aqua dest., 15 min of incubation [8].

The colored gels were densitometered, the running distance between the start of the separating gel and the front band was equalized to 1, and the relative mobilities (R_F values) of the bands were calculated with the utmost accuracy (running dis-

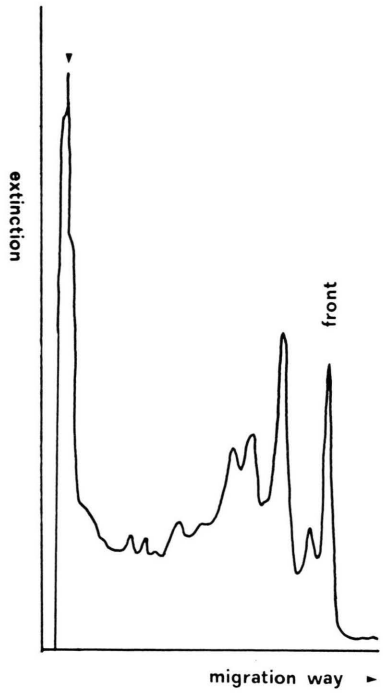

Fig. 3. Electropherogram of a *Chironomus plumosus* homogenate. Amido Black colouring. The arrow marks the beginning of the separating gel.

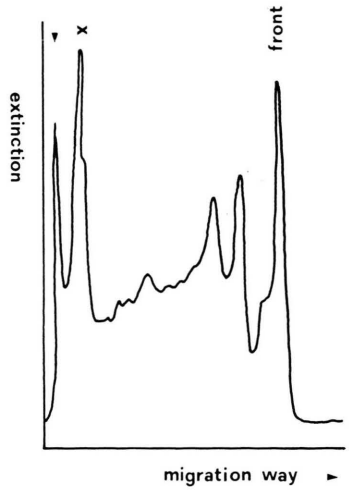

Fig. 4. Electropherogram of a crop content of *Helobdella stagnalis*. The animal has been fed on *Chironomus plumosus*. Amido Black colouring. × marks crop content-specific slowly migrating bands. The other bands have the same R_F values as the bands in homogenate pherograms of *Chironomus plumosus*. The arrow marks the beginning of the separating gel.

tance of the bands concerned, divided by the running distance of the front bands). Means and standard derivations of the R_F values were computed.

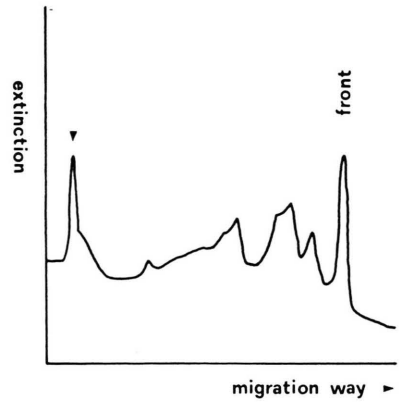

Fig. 5. Electropherogram of a *Chironomus thummi* homogenate. Amido Black colouring. The arrow marks the beginning of the separating gel.

Results

Ch. tentans had three or four bands with R_F values averaging 1.00 ($n=30$), 0.94 ($n=21$), 0.85 ($n=30$), and 0.73 ($n=30$) (Fig. 1 a). Compared with these values *Ch. plumosus* and *Ch. thummi* had five bands each, with R_F values of 1.00, 0.92, 0.82, 0.70, and 0.63 (Fig. 3) or, 1.00, 0.89, 0.81, 0.76, and 0.60, respectively (Fig. 5) (averages of six values of six samples each). Homogenate separations showed the same protein pattern as did hemolymph separations; additionally, however, they showed a sharp peak at the boundary between concentrating and separating gel, facilitating the measuring of the run distance.

In addition to the hemolymph proteins, several slowly migrating bands, varying greatly in number, appeared in the crop contents of *H. stagnalis*, which had been fed the above *Chironomus species* (Figs 2 a, 4, 6). Their R_F values ranged from between 0.06 to 0.30. The variation in number and R_F values rendered homologizing of the bands more difficult. By means of indicatory paper the pH value of the crop contents of *H. stagnalis* was found to be 6.2, the pH of the *tentans* hemolymph 7.1. Homogenates and hemolymphs of *Ch. tentans* showed several esterase isozymes at pH 7.1 (Fig. 1 b). In the separations of the crop contents appeared a fast migrating, intensive esterase band at pH 6.2 (Fig. 2 b). The examination of the whole protein by means of Amido Black 10 B showed these esterases to be completely covered by Chironomus proteins [Keim 1974; unpublished].

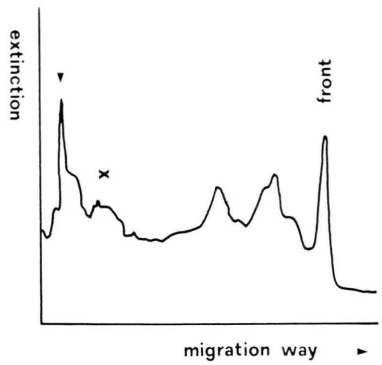

Fig. 6. Electropherogram of a crop content of *Helobdella stagnalis*. The animal has been fed on *Chironomus thummi*. Amido Black colouring. × marks crop content-specific slowly migrating bands. The other bands have the same R_F values as the bands in homogenate pherograms of *Chironomus thummi*. The arrow marks the beginning of the separating gel.

Discussion

Under different conditions (10% acrylamide and 8 mol urea, according to Tichy [9]) *Ch. tentans* had 10 protein bands, appearing partly only in low density. For the objective of this experiment a species characterization could be obtained by means of a few easily recognizable protein bands. The R_F values obtained from the bands were employed for the calculation of the standard deviations from two aspects. First, the R_F values of all *tentans* homogenates, as well as of all crop contents with *Ch. tentans* were computed. Only minor differences appeared between the average values of both groups (0.94 ± 0.03 to 0.95 ± 0.02, 0.86 ± 0.03 to 0.86 ± 0.03, and 0.73 ± 0.02 to 0.74 ± 0.02, (*i. e.*, means $\pm$ standard deviations, respectively). From the values shown it becomes evident that Student's values were in no case significant, with the degrees of freedom not exceding 44. Secondly, the R_F values from only one experiment were statistically analized. The evaluation of the experiment consisting of 15 series of tests with each series being separately computed resulted in standard deviations ranging from 0.003 to 0.016 They must be regarded as very low, the coefficient of variation (CV) being slightly above 2% at its maximum. The differences between the average values of the individual experiments, however, were found to be relatively large, *e. g.*, 0.88 to 0.84 and 0.76 to 0.71. In order to compare the bands, a standard protein must thus be run along with the samples under examination. Unfortunately, however, almost all authors working in this field have neglected to calculate average values and standard deviations. Only Peter [4] gives a standard deviation of 0.006.

The preparation technique applied left us with the eventuality of hemolymph of *H. stagnalis* getting into the samples. An electrophoresis with *Helobdella* hemolymph, however, resulted in entirely different protein bands. If hemolymph *H. stagnalis* contaminates the crop contents during preparation, it may be of an infinitely small quantity as compared with the crop contents, and thus negligible.

For the present, the nature of the crop contents-specific, slowly migrating, proteins may only be speculated on. In identifying the esterase enzymes only low activities were found within the range of these bands. They may be identical with muscle proteins of the prey animals freed by enzymatic tissue-lysis. I have not found any references as to proteolytic processes in the *Helobdella* crop. If *H. stagnalis* is kept at room temperature in the laboratory, the stored food is transported out of the crop into the intestine within two days. The red color of *Chironomus* hemoglobins disappeared in the intestine. The fore-compartment of the crop caeca were first emptied, the intensity of the hemoglobin color being preserved in the crop caeca that has been filled up to that time. Neither intensity diminutions of the bands nor cleavage products from a proteolysis could be detected in the pherograms.

I am grateful to Prof. Schwoerbel for his interest in the subject and his support of this work. Moreover I express my thanks to all who contributed to the success of this work by their help.

[1] J. A. W. Kirsch and M. D. Murray, Aust. J. Zool. **17**, 799–816 [1969].
[2] B. J. Davis, Ann. N. Y. Acad. Sci. **121**, 404–427 [1964].
[3] H. R. Maurer, Disk-Elektrophorese, De Gruyter, Berlin 1968.
[4] R. Peter, Z. zool. Syst. Evolut.-Forsch. **9**, 263–318 [1971].
[5] M. Coates, Evolution **21**, 130–140 [1967].
[6] P. Abramoff, R. M. Darnell, and J. S. Balsano, Amer. Natural. **102**, 555–558 [1968].
[7] H. G. Tunner, Z. zool. Syst. Evolut.-Forsch. **11**, 219–233 [1973].
[8] Ch. R. Shaw and R. Prasad, Biochem. Gen. **4**, 297–320 [1970].
[9] H. Tichy, Chromosoma (Berl.) **29**, 131–188 [1970].