

**ELECTROPHORETIC STUDIES ON THE HAEMOLYMPH PROTEINS IN
PORCELLIO LAEVIS LATREILLE (PORCELLIONIDAE, ISOPODA):
COMPARISON OF THE PATTERNS OBTAINED ON CELLULOSE
ACETATE AND POLYACRYLAMIDE GEL**

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IN recent years zone electrophoresis has been successfully used for rapid characterization of crustacean haemolymph proteins (see References in Alikhan¹, Busselen^{2,3}, Manwell and Baker⁴, and in Wieser⁵). Unfortunately the results obtained by the different electrophoretic techniques are not easily comparable and generally lead to conflicting conclusions³. Haemocyanin, the respiratory protein in most of the species studied, has been reported either to migrate as a single fraction (Alikhan¹, Busselen³, Frentz⁶, Wieser⁵, Zuckerkandl⁷) or to separate into several components (Barlow and Ridgway⁸, Cowden and Coleman⁹, Denucé and Kühn¹¹, Manwell and Baker⁴, Woods *et al.*¹⁰).

The pH-dependence of the electrophoretic pattern of the crustacean haemolymph proteins has been demonstrated by Busselen^{2,3}. The objective of the present note is to show that the variability and complexity of the reported patterns are probably due to the resolution power of the electrophoretic medium employed.

Porcellio laevis adults (tenth instar) used in this study were drawn from a stock colony, maintained on pieces of carrot, in a glass tank at 21° C and 100% r.h. The animals were bled by insertion of a glass capillary tube dorsally through the integument separating the pereopod segments V and VI, and the blood sample, 6.0–10.0 µl/adult, was pipetted into 0.10 ml of 20% glucose solution. In this way the blood could be handled without prior clotting.

Cellulose acetate electrophoresis.—The apparatus used was the Densicord (Photovolt Corporation, New York), and the method described by Wieser⁵, as modified by Alikhan¹, was followed. The following details are of importance: cellulose acetate strips: Gelman Instrument Co.; buffer: Tris-barbital-sodium barbital (Gelman high resolution buffer), pH = 8.5, $\mu = 0.05$; current: 1.2 mA/5 × 10 cm strip; usually four strips were run simultaneously for 3 hr; staining: with Ponceau S (500 mg in 100 ml aqueous solution of 5% trichloroacetic acid), 20 min; scanning: with Densicord after clearing the strips in glacial acetic acid-methanol (1:9, v/v), and drying them at 60° C for 15 min.

Polyacrylamide gel electrophoresis.—The details of the polyacrylamide gel electrophoresis are given by Davis¹², and Maizel¹³. The important details of the procedure are: polyacrylamide gel, 7.5%; buffer: Tris-glycine at pH 8.5; blood sample/gel: 8–10 µl; current: 2–5 mA/5 × 60 mm tube; usually 12 gels were run simultaneously for 1.5–2 hr; staining: with Coomassie brilliant blue R 250; scanning: with EC 910 Transmission Densitometer (E.C. Apparatus Corp.) after several destainings in 7.5% glacial acetic acid. Esterase activity was detected by means of α -naphthyl acetate¹⁴.

The typical electropherograms of the woodlouse blood, as obtained on cellulose acetate and polyacrylamide gel, are presented in Figs. 1 and 2 respectively. The cellulose acetate electropherogram (Fig. 1) shows five bands of various mobilities. Only band 2 reacted positively with rubeanic acid (test for copper) and can therefore be considered to contain haemocyanin. This haemocyanin fraction was always followed by a very small fraction (fraction 5, in Fig. 1), which gave a positive glycoprotein reaction (with P.A.S.). The remaining protein fractions could not be identified, although Tyler and Metz¹⁵ have demonstrated that they may comprise of fibrinogen (fraction 4, Fig. 1), heteroagglutinin (fraction 3, Fig. 1), and apohaemocyanin (fraction 1, Fig. 1).

Compared to the five fractions obtained on cellulose acetate, 7.5% polyacrylamide gel revealed more than seventeen stainable fractions (Fig. 2). For convenience sake, these fractions have been grouped into four major zones: slow proteins (fractions 1–8), haemocyanin (fractions 9–11, identified by the rubeanic acid copper test), esterase (fraction 12), and fast proteins (fractions 13–17). From among the slow proteins, fraction 4 (Fig. 2) was demonstrated to contain lipoprotein (positive reaction with sudan black B), while fraction 6 (Fig. 2) reacted positively with P.A.S., revealing a glycoprotein nature. Fraction 8 in some individuals (in 5 out of 130) showed apohaemocyanin activity. However, it is difficult to say if apohaemocyanin is absent from the blood of the adult. Indeed, in separate experiments apohaemocyanin showed the same electrophoretic mobility on 7.5% polyacrylamide gels as haemocyanin.

The results of this study positively show that the variability of the protein pattern obtained is dependent, to a large extent, on the electrophoretic medium employed. Similar conclusions have been arrived at by Besse and Mocquard¹⁶, Lagarrigue and Trilles¹⁷, and by Romestand¹⁸, who have studied the haemolymph protein pattern in *Sphaeroma serratum*, *Ligia italica*, *Tylos latreillei*, *Halophiloscia couchi*, *Acaeroplastes melanurus*,

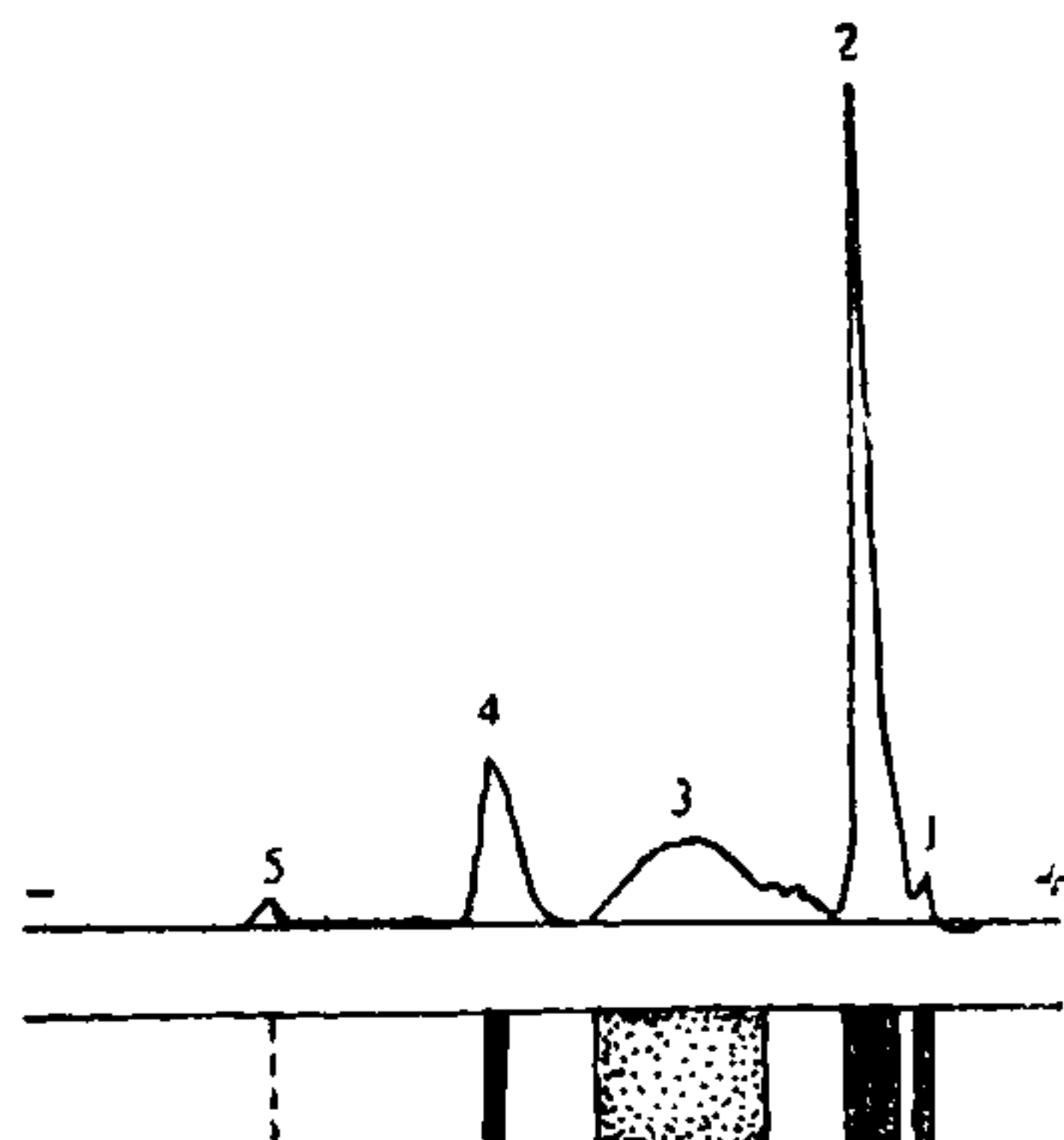


FIG. 1

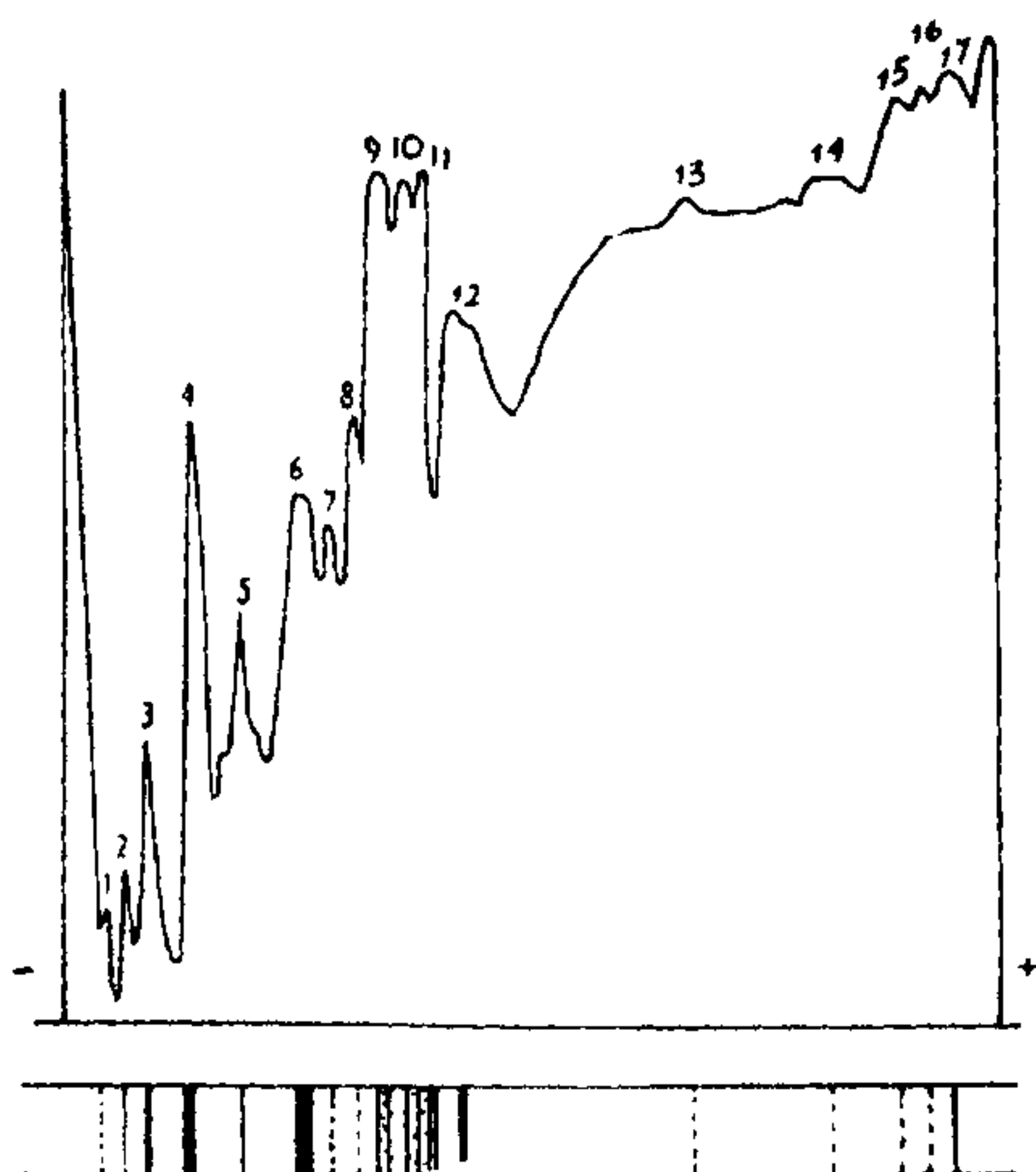


FIG. 2

FIGS. 1-2. Fig. 1. Haemolymph proteins of *Porcellio laevis* (tenth instar) separated by cellulose acetate electrophoresis: 1, apohaemocyanin; 2, haemocyanin; 3, heterogglutinin; 4, fibrinogen; 5, glycoprotein. (Gelman Ponceau stain, optical density). Fig. 2. Haemolymph proteins of *Porcellio laevis* (tenth instar) separated by polyacrylamide gel (7.5%) electrophoresis: Slow proteins (1-8); haemocyanin (9-11); esterase (12); fast proteins (13-17). (Coomassie brilliant blue R 250 stain, optical density).

Porcellio lamellatus sphinx, *Armadillidium vulgare*, and in *Armadillo officinalis*, by cellulose acetate and starch gel electrophoresis.

According to Davis¹², polyacrylamide gel matrix, "unlike other porous media, such as cellulose acetate or starch granules, is a lattice of carbon-carbon polymers with pendant amide group, is relatively inert chemically, and has few or no ionic side groups. Furthermore, the gel is mechanically strong over a wide range of pore sizes (which can be adjusted at will by adjustment of the monomer concentration) and offer kinds of flexibility and versatility not easily attainable with cellulose acetate or starch gels" (page 405). It is, therefore, not surprising that the polyacrylamide gel offers a far superior resolution in the separation of complex protein mixtures, such as blood proteins, than do the cellulose acetate, or starch gels.

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