reduction[6] in KCl. If there is a weak adsorption of any one of the reactants, products or intermediates, the shapes are found to be loop-like[7] (Figs. 2 and 3). However, d.c. and a.c. polarograms of these systems are S-shaped and bell-shaped respectively and these shapes do not indicate the presence of weak adsorption. The effect of weak adsorption is very much evident from Fig. 3, where the loop-like shape at lower frequencies changes to crest-like shape at higher frequencies. The reason for such a change is that there exists an adsorption-desorption equilibrium at low frequencies and with increase in frequency such an equilibrium is not possible for this system owing to the rapid oscillations of a.c. signal compared to the rates of adsorption and desorption. Consequently, the effect due to adsorption on complex plane polarograms is not felt at higher frequencies. The above conclusions are valid for the reduction of *m*-dinitrobenzene also. The effect of the increase in pH is to reduce the role of protonation in the reduction process and at higher pH, the reduction of unprotonated species occurs which is more reversible.

In general, it can be said that the shapes of the complex plane polarograms are greately influenced by the frequency of measurements, reversible-irreversible nature of the systems and other phenomina like adsorption, side reactions, etc. Many a time, from the appearance of the complex plane polarograms, a qualitative idea about the behaviour of the system at electrode interface can be conceived.

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THE PHYLOGENETIC AND ECOLOGICAL IMPLICATIONS OF THE HAEMOLYMPH PROTEINS IN CRUSTACEA

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ABSTRACT

Haemolymph protein patterns have been studied in three crustacean species: the terrestrial herbivorous woodlouse, *Porcellio laevis* (Isopoda), the terrestrial carnivorous pillbug, *Armadillidium vulgare* (Isopoda) and the commonest southern Ontario crayfish, *Orconectes propinquus* (Decapoda).

The studies revealed that the haemolymph protein patterns were more or less identical in *Armadillidium* and *Orconectes*. When the differences due to age, physiological status, sex, diet and season are eliminated, the residual differences, it is postulated, are due to the phylogenetic status of the species.

THE study of naturally occurring haemolymph compounds provides valuable information in the understanding of relationships of one animal to another of the same or different species. Most of the investigations of the crustacean haemolymph proteins have been concerned with the changes occurring in the single species because of its developmental or moulting cycle[1], diet[3], sex[1] and season[6]. Recently, Jażdżewski *et al*[8] reported that, haemolymph protein constituents may be influenced in a species by its habitat. The present investigations, which entails a comparison of the haemolymph protein concentration of three crustacean species should provide valuable information in understanding some aspects of the evolution of these species.

Two isopod species, *Procellio laevis* and *Armadillidium vulgare*, and one decapod species, *Orconectes propinquus*, were used in the study. Haemolymph proteins were separated by polyacrylamide gel electrophoresis method of Davis[5], as modified by Alikhan and Lysenko[1].

Whenever required, the peaks of individual proteins were measured from densitometric tracings of the polyacrylamide gels by means of a polar planimeter. These units were converted to the percentage of the total number of the units for the entire protein pattern,

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and expressed as percentage of the mean total protein of the haemolymph in a normal crustacean adult, as visualized on 7·5% polyacrylamide gel. Protein fractions were identified by the criteria of Alikhan and Lysenko[3].

Typical haemolymph protein patterns of adult crustaceans are depicted in Fig. 1. Almost all haemolymph samples from *Porcellio* showed three bands, while those from *Armadillidium* and *Orconectes* exhibited five and eight bands, respectively, of various mobilities. In *Porcellio*, these bands were comprised of a fast moving haemocyanin, a glycoprotein and a lipoprotein (Fig. 1, A). In *Armadillidium*, the protein bands represented a fast moving and a slow moving glycoproteins, a fast moving and a slow moving haemocyanins and a slow moving lipoprotein (Fig. 1, B). In *Orconectes*, the bands were recognized to be two fast moving lipoproteins, one fast moving haemocyanin, one fast moving glycoprotein, one slow moving haemocyanin, two slow moving glycoproteins and a slow moving lipoprotein (Fig. 1, C).

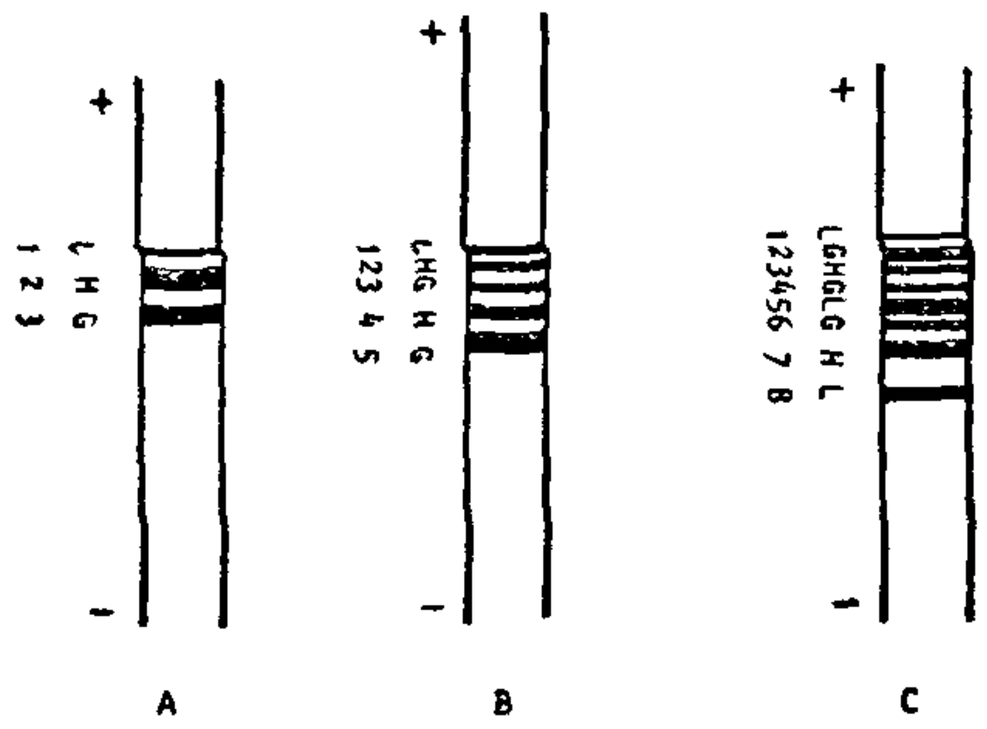

FIG. 1. Electrophoretic pattern of haemolymph proteins, L, liporotein; G. glycoprotein; H, haemocyanin. A. *Porcellio laevis*; B. *Armadillidium vulgare*; C. *Orconectes propinquus*.

In addition, the densitometric tracings of the gels revealed several other unknown proteins. However, since the nature of these proteinic bands could not be detected on the gels by any of the known detection methods, these proteins have been grouped under "others" in Table I, which shows the concentration of the various proteins in the haemolymph of the three crustacean species. An analysis of variance of these data showed that although each species differed from the others in the number of protein bands revealed on the gel, the differences between the amounts of proteins contained in their haemolymph were non-significant.

Relationship Between Haemolymph Protein Pattern and the Sex

No major difference was found in the protein concentrations of the two sexes, except for the concentration of the glycoprotein which was somewhat lesser in the male haemolymph than in that of the female (Table II).

TABLE I

Percentage of detectable proteins, as revealed on 7·5% polyacrylamide gel

	Per cent g/100 ml haemolymph			
	Haemo-cyanin	Glyco-protein	Lipo-protein	Others
Porcellio laevis	20·67	21·95	20·70	36·88
Armadillidium vulgare	19·47	18·37	20·65	41·51
Orconectes propinquus	18·17	16·85	21·57	43·41

Average of 8 replicates in each case.

TABLE II

Variations in protein contents of haemolymph in relation to sex of crustacean

	Per cent g, 100 ml Haemolymph			
	Hemo-cyanin	Glyco-protein	Lipo-protein	Others
Porcellio laevis				
Male	20·30	18·65	20	41·05
Female	20·67	21·95	20·7	36·68
Armadillidium vulgare				
Male	19	16	20	45
Female	19·4	18·30	20·60	41·7
Orconectes propinquus				
Male	19	14	20·90	46·10
Female	18·17	16·80	21·70	43·33

Average of 8 replicates in each case.

Relationship Between Haemolymph Proteins and Nymphal Stage

One way analysis of variance on the data on the haemolymph protein measurements of 72 individuals from the 8th, 9th and 10th instars, showed a significant difference in total ($P > 0·01$), as well as the individual

TABLE III

Variations in the protein contents of haemolymph as a function of the developmental stage of the individual in three different species

	Insta.	Per cent g/100 ml Haemolymph				
		Total	Heamocyanin	Glycoprotein	Lipoprotein	Others
Porcellio laevis	8	89·18	21·52	42·70	6·20	18·76
	9	97·25	20·81	25·32	19·40	31·72
	10	100	20· 7	21·95	20·70	36·68
Armadillidium vulgare	8	82·03	20·31	36·52	5	20·20
	9	86·90	19·47	11·57	18·04	37·82
	10	100	19·47	18·37	20·65	41·51
Orconectes propinquus	8	90·20	24·73	37·90	5·05	22·52
	9	94·45	21·25	11·50	20	41·70
	10	100	18·17	16·85	21·57	43·41

Average of 8 replicates in each case.

TABLE IV

Variations in the protein content of haemolymph in relation to the starvation period

	Fasting Day	Per cent g/100 ml Haemolymph				
		Total	Haemocyanin	Glycoprotein	Lipoprotein	Others
Porcellio laevis	0	100	20·67	21·95	20·70	36·68
	5	100	20·67	21·95	20·70	36·68
	10	67·79	20·67	1·90	20·70	24·50
	15	62·67	19·69	0	18·50	24·50
Armadillidium vulgare	0	100	19·47	18·37	20·65	41·51
	5	100	19·47	18·37	20·65	41·51
	10	75·73	19·47	2·25	18·25	35·76
	15	66·73	18·50	0	15·25	32·73
Orconectes propinquus	0	100	18·17	16·85	21·57	43·41
	5	100	18·17	16·85	21·57	43·41
	10	79·94	18·17	1	21·57	39·20
	15	61·57	15·67	0	16·70	39·20

Average of 8 replicates in each case.

($P < 0{\cdot}01$) protein contents, suggesting a direct relationship between haemolymph protein concentration and the developmental stage age and size/weight of the animal (Table III).

Effect of Experimentally Induced Fasting

As is obvious from Table IV, protein levels in the three species did not change during the first five days of experimentally induced fasting. However, by the tenth day, a sharp and significant decrease in the glycoprotein was noticed in all three species. The concentrations of haemocyanin and lipoprotein were also affected but not to the extent as was glycoprotein which by the 15th day had completely disappeared.

Previous studies[2] have revealed 4 to 17 protein fractions in the crustacean haemolymph. In the present study, interest was centred on only three stainable fractions, namely, haemocyanin, glycoprotein and the lipoprotein. These three protein fractions showed specific differences in terms of their electrophoretic mobilities, but statistically insignificant differences in their concentrations in the haemolymph of the three species. These studies also revealed that the haemolymph protein patterns differ between terrestrial isopods and the freshwater decapod, as well as between the two terrestrial isopod species used. These differences have been related to the physiological differences between these three species[3-6]. When the differences due to age, physiological status, sex, diet and season are eliminated, the residual differences, it is postulated, are due to the phylogenetic status of the species.

In considering the large number of crustacean species, one finds that very little has been done towards devising a taxonomic formula based on electrophoretic separation of proteins. Electrophoretic techniques have been employed for this purpose only a few times and that also with varied results. Electrophoretic analysis of proteins has, at times, falsely led investigators to imply that identically located bands are identical proteins. It is suggested that similar band patterns in closely related species may be due to homologous proteins.

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CADMIUM SEMINAR ON 26TH FEBRUARY 1980 AT UDAIPUR

The seminar is organized by the Indian Lead Zinc Information Centre, New Delhi, in co-operation with Hindustan Zinc Limited, Udaipur, and Comnco Binani Zinc Limited, Alwaye, with the objective of creating an awareness among the Indian Industry of the existing and potential uses of the metal and informing about the environmental aspects, in addition to reviewing the status of cadmium in the Indian and global context. Technical papers and/or notes dealing with production, properties and applications of cadmium will be presented and discussed at the seminar. Contributed papers from experts in the field, relating to major application areas such as cadmium in PVC as stabilisers, in paints as pigments, in batteries, in alloys and in plating and ceramics will be presented and fully discussed. For details, please contact Sri V. R. Subramanian, General Manager, Indian Lead Zinc Information Centre, B-6/7, Shopping Centre, Safdarjung Enclave, New Delhi 110 016.