PHYSIOLOGY OF HOST PARASITE RELATIONSHIPS OF *PALLISENTIS NAGPURENSIS* AND *SENGA VISAKHAPATNAMENSIS*, PARASITIZING LIVER OF *COLISA LALIA* AND INTESTINE OF *CHANNA PUNCTATUS*

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BHALERAO[1] described *Pallisentis nagpurensis* from *Channa striatus*. George and Nadakal[2,3] elucidated its life cycle, incriminating cyclops as intermediate and *Colisa lalia (Trichogaster fasciatus)* as transport hosts. They described encapsulation of immature juveniles in the liver of definitive and transport hosts.

The present authors have recovered *P. nagpurensis* from *C. punctatus* and encapsulated juveniles from *C. lalia*. Every one of the 1172 *C. lalia*, examined from January 1981 to December 1982, was found infected, harbouring 1 to 10 encapsulated juveniles. Curiously, they also harboured, concurrently, encapsulated plerocercoids of *Senga visakhapatnamensis* Ramadevi and Rao, 1973. Thus, at Raipur, *Channa punctatus* is the definitive and *Colisa lalia* the transport host of both *P. nagpurensis* and *S. visakhapatnamensis*. Paradoxically, while the adult worms of the two species negatively interacted in the intestine of *Channa punctatus*[4] their larvae invariably occurred concurrently in the liver of *C. lalia*.

Hardly anything is known of their host-parasite relationships. The present authors have studied certain aspects of their protein metabolism *vis-a-vis* the host tissue to assess inter specific and stage specific differences and parasitic effects on physiology of host tissue.

Encapsulated juveniles of *P. nagpurensis* and encapsulated plerocercoids of *S. visakhapatnamensis* were separately harvested from the liver of *C. lalia*, washed with distilled water, dried on Whatman filter paper and teased. Capsule wall and juveniles or plerocercoids were separated. Pieces of liver, from the infected and the uninfected *C. lalia* and adult worms of the two species, from intestine of *C. punctatus*, were likewise washed with distilled water and dried on Whatman filter paper. Tissues were later dried in an oven at 80°C to constant weight. Homogenates of fresh samples were prepared in normal ice-cold saline, centrifuged at 3000 rpm for 15 min at 4°C and the supernatants were used for enzyme study.

Equal weights of dried tissue of capsule wall, juveniles, plerocercoids, mature worms, and liver (from both infected and uninfected host) were taken for extraction of the free amino acids in 70% ethanol. Proteins from different tissues were precipitated with 14% TCA and purified by removal of fats in acetone, followed by ether; phospholipids in a chloroform: methanol (1 : 1) mixture at 55°C and nucleic acids in 7% TCA. Purified protein was then dried and subjected to hydrolysis employing 6 N HCl and 6 N NaOH at 120°C for 8 hr. HCl and NaOH extracts were later evaporated and the hydrolysates diluted appropriately with 70% ethanol. Both free and protein amino acids were detected by uni- and two-dimensional paper and thin layer chromatography. Identification was achieved by comparing the R_f values with the standards developed under identical conditions. They were later eluted in 70% methanol and estimated colorimetrically at 570 nm using leucine as standard. Total free amino acids and proteins were estimated following usual methods[5,6].

Relative activities of transaminases, phosphomonoesterases and acetylcholinesterase in supernatants were determined colorimetrically following published methods[7–9]. Protein in homogenate was estimated by Lowry's[6] technique using bovine serum albumin as the standard.

Decrease in the total protein and relative activity of transaminases and increase in cholinesterase and non specific phosphomonoesterases activity followed infection of *C. lalia* and, further, while polar negatively charged amino acids constituted as much as 40% of free amino acid pool of uninfected host liver, non-polar amino acids dominated the free pool of infected host liver. Infection clearly led to a change in host liver physiology. Capsule wall of juveniles of *P. nagpurensis* and plerocercoids of *S. visakhapatnamensis* were obviously the result of inflammatory reaction. Developing worms inside capsules, however, continue to obtain nourishment from host liver through the wall of capsule[10–12]. Hydrolytic activity is naturally augmented following stressed metabolism of host tissue due to infection. Increase in the percentage composition of non-polar amino acids in free pool of host tissue, following infection, may, in all likelihood, be due to worms excreting them. Parasites are known to excrete amino acids[13–15].

Mature worms of *P. nagpurensis*, from the intestine of *C. punctatus*, revealed higher levels of protein, total free amino acids and GOT activity; capsule wall and juveniles (from liver of *C. lalia*), however, showed much higher activity of GPT, acetylcholinesterase and non-specific phosphomonoesterases (table 1) suggest-

Table 1 *Total free amino acids, protein and activities of transaminases, non-specific phosphomonoesterases and acetylcholinesterase of P. nagpurensis (adult), juvenile, capsule wall, S. visakhapatnamensis (adult), plerocercoid, capsule wall, uninfected and infected liver of C. lalia (values mean ± standard deviation).*

	P. nagpurensis			*S. visakhapatnamensis*			Liver	
	Adult	Juvenile	Capsule wall	Adult	Plerocercoid	Capsule	Uninfected	Infected
I Total free amino acids (mg/100 mg)	1.04 ±0.21	0.76 ±0.13	0.87 ±0.15	1.19 ±0.35	1.31 ±0.43	1.94 ±0.46	2.30 ±0.88	2.47 ±0.64
II Total protein (mg/100 mg)	38.28 ±1.56	28.60 ±4.15	27.49 ±1.59	32.65 ±3.24	13.09 ±2.62	50.47 ±2.95	42.28 ±2.39	32.85 ±2.55
III Transaminases (μg pyruvate/mg protein/hr)								
i. GOT	90.19 ±14.20	47.34 ±10.41	63.29 ±25.35	231.39 ±38.20	62.48 ±18.27	60.52 ±12.58	70.97 ±6.15	45.58 ±2.96
ii. GPT	16.10 ± 7.40	44.30 ±22.85	46.74 ± 2.96	113.54 ±25.10	7.96 ± 3.15	21.05 ± 9.22	48.81 ±6.15	22.46 ±2.93
IV. Phosphomonoesterases (μg pi/mg protein/hr)								
i. ACP	7.14*	20.00 ± 7.45	12.50*	40.34 ± 3.67	22.61 ± 9.81	72.50 ±10.45	28.23 ±1.61	34.09 ±3.46
ii. AKP	10 85 ± 3.58	29.99 ± 7.45	15.00 ± 5.59	558.26 ±34.87	31.99 ±12.15	131.25 ±21.65	72.94 ±2.46	114.61 ±4.93
V. Acetylcholinesterase (μg acetylcholine hydrolysed/mg protein/hr)	0.38 ±0.08	0.56 ±0.11	0.67 ±0.21	—	0.74 ±0.07	0.17 ±0.06	0.37 ±0.05	0.55 ±0.04

* Based on single observation. GOT = glutamate oxaloacetate transaminase. GPT = glutamate pyruvate transaminase. ACP = acid phosphatase. AKP = alkaline phosphatase.

Table 2 *Free amino acids (in percent) of P. nagpurensis (adult), juvenile, capsule wall, S. visakhapatnamensis (adult), plerocercoid, capsule wall, uninfected and infected liver of C. lalia.*

Amino Acids	*P. nagpurensis*			*S. visakhapatnamensis*			Liver	
	Adult	Juvenile	Capsule wall	Adult	Plerocercoid	Capsule wall	Uninfected	Infected
1 + 2 Leucine + Iso-leucine	11.01	6.24	4.34	12.31	16.21	7.39	5.05	16.06
3. Phenylalanine	1.32	1.96	2.02	1.33	1 64	1.15	0.37	1.54
4. Valine	6.68	4.52	5.74	6.19	10.24	9.17	2.13	7.20
5. Methionine	1.04	—	—	0.96	1.07	2.63	—	—
6. Tyrosine	1.32	1.96	10.05	1.21	0.93	1.37	0.75	1.77
7. α-amino-*n*-butyric acid	4.98	—	—	—	—	—	—	—
8. Proline	8.30	13.63	6.89	12.61	4.49	4.14	2.99	4.17
9 Alanine	15.82	7.57	4 82	17.99	16.67	11.45	18.19	20.18
10. Threonine	1.04	1.70	5.12	2.43	2.90	4.55	3.36	3.71
11. Glutamic acid	6.50	9.32	8.27	10.07	8.19	9 41	17.81	7.75
12. Glycine	10.83	8.52	23.18	6.06	8.12	10.24	7.87	5.40
13. Aspartic acid	4.80	6 19	4.61	3.53	4.61	11.55	21.38	9.32
14. Arginine	2.35	2.24	3.45	2.92	1.48	5.07	2.80	3 20
15. Serine	1.04	2.55	1.72	3.65	4.50	1.89	2.62	—
16. Histidine	3.49	6.22	5.99	4.25	4.14	5.62	2.62	6.35
17. Ornithine	5.46	9.56	13.72	5.96	6.84	12.31	5.61	9.15
18. Lysine	2.07	1.96	—	1.81	1.59	—	2.62	—
19. Cystine	—	2.82	—	—	—	—	—	2 10
Unidentified	11.86	12.95	—	6.80	6.27	2.01	3.78	2.02
Amino acids when classified according to their charge:-								
Non polar	49.15	33.92	23.81	51.39	50.32	35.91	28.73	49 15
Polar (neutral)	14.23	17.55	40.07	13.35	16.45	18.05	14.60	12.98
Polar (+vely charged)	13.37	19.98	23.16	14.94	14.05	23.00	13.65	18.70
Polar (−vely charged)	11.30	15.51	12 88	13.60	12 80	20.96	39.19	17.07

ing extensive hydrolytic activity, active uptake and transamination, all so necessary for the developing juveniles and in conformity with the suggestion made earlier that developing worms continue to obtain nourishment from host liver through capsule wall. Occurrence of α-amino-*n*-butyric acid in both free and protein pool of mature worms of *P. nagpurensis* (cf. capsule wall and juvenile where it is absent from both free and protein pools) is possibly stage-specific. (tables 2, 3).

Mature worms of *S. visakhapatnamensis*, from the intenstine of *C. punctatus*, showed very high activity of both transaminases and alkaline phosphatase. Protein levels, however, were found highest in capsule wall (table 1). Differences were also noticeable in protein composition. α-amino-*n*-butyric acid appeared characteristically in protein pool of plerocercoid. Polar negatively-charged amino acids contributed substantially more to protein pool of mature worms (table 3).

In conclusion, species of worms, although parasitizing a common habitat concurrently, exhibit fairly biochemical individuality in their metabolism and protein profiles. Besides, they manifest stage-specific biochemical differences. Negative interaction between *P. nagpurensis* and *S. visakhapatnamensis* in the intestine of *C. punctatus* may possibly be due to non-reciprocal cross immunity[16-19]. In the liver of *C. lalia*, however, (since worms of both species are encapsulated) there would be very little of competition, hence invariably occur together.

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Table 3 *Protein amino acids (in percent) of P. nagpurensis (adult), juvenile, capsule wall, S. visakhapatnamensis (adult), plerocercoid, capsule wall, uninfected and infected liver of C. lalia.*

	P. nagpurensis			*S. visakhapatnamensis*			Liver	
Amino Acids	Adult	Juvenile	Capsule wall	Adult	Plerocercoid	Capsule wall	Uninfected	Infected
1 + 2 Leucine + Iso-leucine	24.43	14.95	18.95	8.47	18.25	16.70	26.28	19.28
3. Phenylalanine	3.74	1.79	1.22	1.54	1.35	1.70	2.67	2.05
4. Valine	9.76	8.53	5.98	11.31	11.55	6.83	5.88	9.02
5. Methionine	3.36	2.16	1.22	3.03	1.46	1.59	0.94	1.40
6. Tyrosine	2.18	2.07	1.25	1.92	1.18	1.72	1.54	1.93
7. α-amino-*n*-butyric acid	0.32	—	—	—	0.22	—	0.31	0.18
8. Proline	2.83	1.18	2.48	1.83	2.81	0.73	2.22	1.95
9. Alanine	15.45	13.65	12.47	13.39	16.04	13.58	20.37	14.22
10. Threonine	3.94	5.59	5.17	7.67	5.60	6.16	5.24	4.83
11. Glutamic acid	4.29	12.40	11.31	16.52	10.58	10.45	2.65	11.97
12. Glycine	5.53	5.59	6.83	6.36	7.02	6.44	4.42	5.15
13. Aspartic acid	4.59	7.90	7.06	6.53	4.39	7.55	4.16	6.38
14. Arginine	4.75	6.34	6.94	5.38	6.79	9.49	3.55	4.41
15. Serine	1.17	1.57	1.02	3.25	0.96	1.42	1.23	3.40
16. Histidine	5.26	7.85	6.61	5.26	4.15	6.88	5.64	5.36
17. Ornithine	5.22	6.21	9.40	6.26	6.93	6.96	11.32	6.20
18. Lysine	0.55	0.68	0.64	0.70	0.45	0.95	0.68	1.05
19. Cystine	—	—	—	—	—	—	—	—
Unidentified	2.45	1.45	1.39	0.52	0.18	0.83	0.92	1.13
Aminoacids when classified according to their charge:-								
Non polar	59.89	42.26	42.29	39.57	51.68	41.13	58.67	48.10
Polar (neutral)	12.82	14.82	14.27	19.20	14.76	15.74	12.43	15.31
Polar (+vely charged)	15.78	21.08	23.59	17.60	18.32	24.38	21.09	17.02
Polar (−vely charged)	8.88	20.30	18.37	23.05	14.97	18.00	6.81	18.35

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MORPHOLOGY OF THREE SPECIES OF CONCHOSTRACA USING SCANNING ELECTRON MICROSCOPE

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IN taxonomic studies of Conchostraca, morphological characters have, in most instances, provided the body