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Some Aspects of Haematology of an Air Breathing Indian Mud Eel, *Amphipnous cuchia*

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Abstract In comparison with other freshwater teleosts, *Amphipnous cuchia* showed lower pH value (about 7.3) and higher specific gravity (about 1.08) of the blood, and similar level of protein, glucose, and other plasma components. Only six amino acids, alanine, arginine, glycine, histidine, leucine, and methionine, were separated from the plasma.

The suborder Synbranchoidei of order Synbranchiformes comprises two families viz., Synbranchidae and Amphipnoidae represented by eel-shaped fish with a tapering tail and greatly reduced fins.

Recently, Rosen and Greenwood (1976) revised the systematics of Synbranchiformes, and classified genera *Amphipnous*, *Fluta*, *Synbranchus* and others into the subfamily Synbranchinae. As regards the genus *Amphipnous*, they synonymized *Amphipnous* with *Monopterus*, i.e., the scientific name of the mud eel changes from *A. cuchia* to *M. cuchia*.

Amphipnous cuchia (Hamilton) is found in holes and crevices in the muddy banks of marshes and slow running rivers (Hora, 1935) of fresh and brackish waters of India, Pakistan, Bangla desh and Burma. The gills of *Amphipnous* are greatly reduced except a few coarse filaments on the second branchial arch (Munshi and Singh, 1968; Hughes and Munshi, 1973). Aquatic respiration through the skin is supplemented by aerial respiration through air breathing organs in the form of a pair of sacs on lateral side of the head. Although this fish is adapted to extremely droughty conditions, water remains as its primary medium for normal metabolism. Recent studies on the control of breathing in *Amphipnous cuchia* suggest that this fish has retained the piscine mechanism of ventilating the rudimentary gills with water (Lomholt and Johansen, 1974b). Active branchial respiration of water never occurs unless the fish is deprived of access to air. Oxygen extraction from water ventilation varied in experimental condition between 10～25% (Lomholt and Johansen, 1974b).

The morphology and anatomy of air breathing organs of *Amphipnous cuchia* has been worked out by Munshi and Singh (1968) and Hughes and Munshi (1973), but the respiratory physiology and haematological parameters are still obscure and restricted to few works (Srivastava, 1968; Hughes et al., 1974; Lomholt and Johansen, 1974a, 1974b; Mishra et al., 1977). Therefore, the present investigation on *Amphipnous cuchia* has been undertaken for securing biochemical scores of plasma, to be helpful in evaluating physiological status of the animal.

Materials and methods

Sexually adult fishes of various length and weight of either sexes were collected in the months of May to June, 1975 from the ponds and swamps of Alamnagar (Dist.-Saharsa, Bihar) and were maintained in aquaria in the laboratory at Bhagalpur. The fishes were fed with earthworm and pieces of properly cleaned intestine of goat. After proper acclimatization of the animals in laboratory conditions, i.e., three weeks from the date of capture, blood was taken out directly from the ventricle with hypodermic needle (No. 22) using heparin as an anticoagulant. Aliquots of whole blood was used for electrometric determination of blood pH through micro-glass electrode and specific gravity of blood was determined by copper sulphate method after Phillips et al. (1950). Plasma was obtained after centrifugation of the blood and was further analysed on the same day.

Total plasma protein was determined colorimetrically by Biuret method (Gornall et al., 1949) and plasma glucose by anthrone method (Seifter et al., 1950). Total, esterified, and free

Table 1. Values of certain blood/plasma parameters of *Amphipnous cuchia*.

Sex (Number of fish)				Male (17)	Female (16)
Body weight (g)			Mean±S.D. Range	400.118±92.58 260.0~597.0	382.625±94.90 219.0~586.0
Gonado-somatic index			Mean±S.D. Range	0.163±0.047 0.099~0.268	1.387±0.890 0.268~2.716
Blood pH			Mean±S.D. Range	7.29±0.006 7.20~7.41	7.26±0.080 7.15~7.45
Specific gravity of blood			Mean±S.D. Range	1.079±0.011 1.063~1.100	1.083±0.10 1.069~1.108
Plasma components	Protein (g/100 ml)		Mean±S.D. Range	3.304±1.106 1.777~6.221	3.745±0.850 2.666~7.110
	Glucose (mg/100 ml)		Mean±S.D. Range	67.344±17.694 32.413~103.720	72.468±27.139 38.031~144.773
	Triglyceride (mg/100 ml)		Mean±S.D. Range	257.027±84.330 136.114~418.127	224.747±78.210 146.762~470.536
	Cholesterol	Total (mg/100 ml)	Mean±S.D. Range	216.038±86.100 102.408~508.600	307.000±106.334 111.892~508.600
		Esterified (mg/100 ml)	Mean±S.D. Range	137.105±58.266 71.204~330.590	196.328±70.69 73.324~345.848
		Free (mg/100 ml)	Mean±S.D. Range	78.933±31.846 30.516~178.010	110.672±42.92 37.981~160.320

cholesterol in plasma was determined according to Webster (1962) and triglyceride by colorimetric method of Carlson (1963). Plasma amino acid composition of a few fishes were analysed according to Brenner et al. (1965) through thin-layer chromatography using silica gel.

In the present investigation, 17 male and 16 female fish having a mean body weights of 400.118±92.58 g and 382.625±94.90 g respectively were used. During experiments the average water temperature was 31.2±1°C.

Results and discussion

Blood pH. Literature on blood pH of fishes are few and show great diversity of values ranging between 7.0 to 8.0. The blood of *Amphipnous cuchia* is more towards neutral point (Table 1) than those of *Ictalurus*, *Squalus* and *Salvelinus* species (Haws and Goodnight, 1962; Maren, 1962; Hoffert and Fromm, 1966). The low blood pH of *Amphipnous* should be considered as one of the adaptations related to the ecological conditions. Similar shift of blood pH towards acidity has also been reported in many reptiles (Dessauer, 1970). Rahn (1967) and Garey (1967) observed that in poikilotherm vertebrates the blood pH varied inversely with body temperature. In reptiles during diving and burrowing, a sharp drop in blood pH has been observed with increasing CO_2-tension, rise in lactate concentration and marked decrease in bicarbonate level of blood (Dessauer, 1970). As *Amphipnous cuchia* has burrowing habit and can withstand extremely droughty conditions, it seems probable that the adaptation to high temperature together with burrowing habit in which the animal is exposed to hypoxic to anoxic condition for prolonged periods, shifted the blood pH of this animal towards acidic. However, the role of electrolyte, renal function and the mechanism of its control need detailed investigations.

Specific gravity. The specific gravity of blood in male and female *Amphipnous* was found to be 1.079 and 1.083 respectively (Table 1). Haws and Goodnight (1962) obtained blood specific gravity of 1.035 in *Ictalurus punctatus* and 1.034 in *Ictalurus nebulosus*. The specific gravity of blood of closely related species may not show much difference, but the fish belonging to different genera and families of various ecological niches may show variation in this parameter.

Protein. Informations available on the amount and nature of plasma protein in fishes are fragmentary and scattered (Satchell, 1971).

Table 2. Free amino acids of human, alligator, and fish blood plasma as reported by various authors. +, indicates presence; −, indicates absence/not reported.

Amino acid	Mammal	Reptile	Fish		
	Human beings (West et al., 1970).	Alligator (Coulson and Hernandez, 1965)	*Oncorhynchus tshawytscha* (Chance, 1962)	*Cyprinus carpio* (Creaćh and Serfaty, 1964)	*Amphipnous cuchia* Present report
Alanine	+	+	+	+	+
L-Aminobutyric acid	+	−	−	−	−
Arginine	+	+	+	+	+
Asparagine	+	−	−	−	−
Aspartic acid	+	+	+	+	−
Citrulline	+	+	−	−	−
Cystine	+	−	−	+	−
Glutamic acid	+	+	−	+	−
Glutamine	+	+	−	−	−
Glycine	+	+	−	+	+
Cystathiomine	−	−	+	−	−
Histidine	+	+	+	+	+
Isoleucine	+	+	+	+	−
Leucine	+	+	+	+	+
Lysine	+	+	+	+	−
Methionine	+	+	+	+	+
Ornithine	+	+	−	−	−
Phenylalanine	+	+	+	+	−
Proline	+	−	−	+	−
Serine	+	+	+	+	−
Taurine	−	−	+	−	−
Threonine	+	+	+	+	−
Tryptophan	+	−	−	−	−
Tyrosine	+	+	+	+	−
Valine	+	+	+	+	−

The plasma protein content of *Amphipnous* (Table 1) is of the same range as in many other teleosts, e.g., *Clarias gariepinus* 3.23 g% (Hattingh, 1974a), *Labeo umbratus* 3.03 g%, *Labeo capensis* 3.59 g% (Hattingh, 1974b), *Cyprinus carpio* 3.60 g% (Pandey, 1974), and *Catla catla* 3.11 g% (Das, 1961). In the present investigation differences in plasma protein of both sexes were observed (Table 1) although this difference was not statistically significant. Several evidences of hormonal response of plasma protein in fishes are indicated (Barr, 1965; Hunn, 1967; Hawkins and Mawdesley-Thomas, 1972).

Amino acids. Amino acids can be effectively separated into various fractions upon ion-exchange chromatography. Chance (1962) in *Onchorhynchus tshawytscha* and Creaćh and Serfaty (1964) in *Cyprinus carpio* separated more than 15 amino acids from plasma. In the present investigation we could locate only six amino acids (Table 2) in the plasma of *Amphipnous* using n-Butanol-acetic acid-water (v/v 80 : 20 : 20) as solvent on thin-layer silica gel. Besides other amino acids, alanine, arginine, histidine, leucine, and methionine are common constituents of plasma proteins of fishes (Table 2), reptiles (Coulson and Hernandez, 1965), and mammals (West et al., 1970). Creaćh and Serfaty (1964) found cystine and ornithine to be present only in traces in plasma of *Cyprinus carpio*. Citrulline is found in human plasma (West et al., 1970) but not reported from fish plasma. Glycline is found in *Cyprinus* and *Amphipnous* (Table 2) but is absent in Chinook salmon (*O. tshawytscha*). The role of these amino acids in the metabolism of fish is not exactly known.

Glucose. Glucose analysis are commonly carried out on blood rather than plasma,

Table 3. Values of blood or plasma glucose level of certain air breathing fishes of India.

Species	Methods of analysis	Glucose (mg/100 ml)	Reference
Notopteridae			
Notopterus notopterus	Follin-Wu, 1920	87.0±13.01 (Blood)	Sunderaraj et al., 1966
Clariidae			
Clarias batrachus	Nelson-Somogyi, 1944	63.3±11.7 (Blood)	Joshi, 1974
Clarias batrachus	Nelson-Somogyi, 1944	60.5±1.30 (Plasma)	Siddiqui, 1974
Heteropneustidae			
Heteropneustes fossilis	Nelson-Somogyi, 1944	76.0±1.91 (Plasma)	Siddiqui, 1974
Heteropneustes fossilis	—	84.0±4.33 (Blood)	Khanna and Rekhari, 1972
Channidae			
Ophiocephalus punctatus	Nelson-Somogyi, 1974	50.0±1.58 (Plasma)	Siddiqui, 1974
Ophiocephalus striatus	Nelson-Somogyi, 1974	42.0±0.99 (Plasma)	Siddiqui, 1974
Synbranchidae			
Amphipnous cuchia (Male)	Anthrone method	67.344±17.694 (Plasma)	Present report
Amphipnous cuchia (Female)	Anthrone method	72.468±27.139 (Plasma)	Present report

although McCay (1931) and Andreen-Svedberg (1933) reported absence of glucose mostly from erythrocytes. Blood or plasma glucose in fishes has been reported by various authors (Love, 1970), the values of which show wide range of variations. Attempts have been made to interpret blood glucose level with the biology and adaptive physiology of fishes. Leibson (1972) concluded that active marine teleosts differ from sluggish ones by comparatively high blood glucose. Blood glucose has been further accessed as a sensitive indicator of environmental stress caused by pollution (Johansson et al, 1972; Silbergeld, 1974).

The blood or plasma glucose level in air breathing fishes as reported by various authors including the present ones has been compared in table 3, a perusal of which indicates that the plasma glucose level in *Amphipnous* is in agreement with other air-breathing fishes of India. In *Amphipnous* great individual variations in the glucose level (Table 1) were observed. As this is a feral fish, it is not known whether these fluctuations are due to captivity or related to other factors. According to Young (1974) blood carbohydrate levels in vertebrates is influenced by numerous factors including analytical technique, strain, sex, age, nutritional status, enviromental conditions, anaesthesis, methods of handling animals etc.

Cholesterol. Much higher values of total plasma cholesterol (300~700 mg%) from certain teleosts have been reported (Sulya et al., 1960; Lewander et al., 1974; Shibata et al., 1974). The total plasma cholesterol of *Amphipnous* is lower than *Anguilla anguilla* (see Larsson and Fänge, 1969). In female, total plasma cholesterol level was higher than in male, but the percentage of esterified and free cholesterol remained same. The plasma triglyceride content of this fish is higher than in mammals but is in agreement with the findings of Shibata et al. (1974) in rainbow trout and Lewander et al. (1974) in European eel.

The plasma biochemical scores of *Amphipnous* with some exceptions are much similar to such migrating fishes as eel and trout. In order to understand mobilization of metabolic energy of this fish, it is essential to examine other organs besides blood, as it is supposed that in fish there is direct utilization of fat reserves for metabolic energy without participation of blood stream (Larsson and Fänge, 1969).

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空気呼吸をするインド産タウナギ類の1種 *Amphipnous cuchia* の血液性状

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他の淡水産硬骨魚類と比較して, *Amphipnous cuchia* の血液 pH 値はやや低く (約 7.3), 血液比重はやや高い (約 1.08) が, タンパク質, グルコースその他の血漿成分濃度はほぼ等しかった. 血清アミノ酸の種類は, コイやマスノスケより数少なく, アラニン, アルギニン, グリシン, ヒスチジン, ロイシンおよびメチオニンの6種であった.