ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XIII Fasc. 2 Szczecin 1983

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Fish pathology

GYMNORHYNCHUS GIGAS PLEROCERCOID (*CESTODA: GYMNORHYNCHIDAE*) INFECTION OF THE LIVER OF *DIODON HYSTRIX* *(PISCES: DIODONTIDAE)*

III. BIOCHEMICAL COMPOSITION OF INFECTED FISH

ZARAŻENIE WĄTROBY *DIODON HYSTRIX (PISCES: DIODONTIDAE)* PRZEZ PLEROCERKOIDY *GYMNORHYCHUS GIGAS (CESTODA: GYMNORHYNCHIDAE)*

III. BIOCHEMICZNE ZMIANY U ZARAŻONYCH RYB

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The changes in the proximate composition of the liver and white muscle of *Diodon hystrix* infected with the plerocercoid of *Gymnorhynchus* gigas have been studied. The changes in the liver are marked by asignificant increase in water content, free fatty acid and total nitrogen contents concurrent with a significant decrease in fat and glycogen. In the case of muscle the most conspicuous change is the significant reduction of glycogen content and significant increase in water and free fatty acid contents. In general, the changes in the proximate composition brought about by the infection are comparable to those occurring during starvation.

INTRODUCTION

Only very little information is available on the changes in the proximate composition of fish tissue in relation to diseases. The few available publications are those of

Meyer-Waarden and Mann (1950).), Petrushevski and Kogteva (1954), Petrushevski and Shulman (1955), Kosheva (1956), Kabata (1958), Mann (1960a,b, 1964, 1965), Abrosov et al. (1963), Shpolyanskaya (1963), and Reichenbach-Klinke et. al (1968), who have studied the fat content of liver/muscle of parasitised fishes. The only publication which has dealt with the water, fat, protein and glycogen content of the liver, muscle and gonad of *Hemirhampus xanthopterus* infected with *Lernaeenicus hemirhamphi* is that of Natarajan and Nair (1976).

In the present paper an attempt has been made to describe the changes in the proximate composition of the liver and muscle of *Diodon hystrix* infected with the plerocercoid of *Gymnorhynchus gigas*.

MATERIALS AND METHODS

Host fish collected from shore seines operating at Shankumughom and Valiyathurai, Trivandrum, were transported alive to the laboratory in large containers. The fish were marked with identifying labels and samples were prepared for biochemical studies. The analyses were carried out on fish ranging from 13.5 to 15.5 cm in standard length, in which length group the incidence of infection is high (see Radhakrishnan and Nair, 1980). Thirty uninfected and thirty infected fish (15 males and 15 females) were used for the analyses.

Water content of the liver and muscle was determined by the method suggested by Suryanarayanan and Alexander (1972) and fat content by the Soxhlet-ether extraction technique. AOAC (1945) method was employed for estimating free fatty acid content. Glycogen content was determined by titrimetric method suggested by Hagedorn and Jensen (1923) cited by Lange (1967).

Total protein content was calculated from total nitrogen content estimated by Kjeldahl (semimicro) method (Oser 1976). Similarly non-protein nitrogen and nitrogenous waste nitrogen were also estimated by the methods suggested by Oser (1976).

RESULTS

From the results presented in Table 1 it is clear that the conspicuous change in the proximate composition of the liver of infected fish is the increase in water content ($p < 0.001$) and a concurrent decrease in fat ($p < 0.001$). Free fatty acid content in the infected liver is higher than that in the uninfected ($p < 0.001$). Glycogen content in the infected liver is only 41.6% of that in the uninfected. The total nitrogen content of the infected liver is significantly higher than that in the uninfected liver ($p < 0.001$). It is also clear that this observed increase in total nitrogen content is the result of the increase both in protein nitrogen and non-protein nitrogen. Increase in non-protein nitrogen content of the infected liver is seen to be the result of an increase in the peptide-amino nitrogen.

Table 1

Mean (M), standard error (S.E.) and range (R) of the proximate composition of the liver and white muscle of uninfected and infected (*G. gigas* plerocercoid infection) *Diodon hystrix*

Proximate composition +		Liver			Muscle		
		Uninfected fish	Infected fish	t value	Uninfected fish	Infected fish	t value
Water	M	38.80	61.70	17.5792***	73.66	77.69	3.9192***
	S. E.	±1.15	±0.61		±0.75	±0.70	
	R	30.1–51.7	55.5–68.7		67.1–85.0	70.0–84.2	
Fat	M	51.15	35.93	12.4764***	6.09	6.48	0.7544
	S. E.	±1.14	±0.62		±0.50	±0.13	
	R	40.0–64.3	29.5–42.7		1.3–11.0	5.2–8.4	
Free fatty acid	M	398.61	434.20	6.5372***	119.10	128.44	3.2832**
	S. E.	±4.28	±3.36		±2.05	±1.98	
	R	355.7–445.5	396.6–456.8		98.7–139.7	109.8–145.8	
Glycogen	M	11.30	4.70	20.0785***	20.65	11.65	11.5186***
	S. E.	±0.27	±0.17		±0.70	±0.35	
	R	8.0–12.8	3.0–6.4		14.2–28.5	7.3–15.0	
Total nitrogen	M	65.57	71.25	8.0785***	65.09	65.89	0.5319
	S. E.	±0.61	±0.43		±0.99	±1.10	
	R	58.0–70.5	65.0–76.0		54.1–74.0	49.8–73.9	
Protein nitrogen	M	51.90	55.27	2.0790*	57.33	56.80	0.4053
	S. E.	±1.46	±0.71		±0.77	±1.01	
	R	32.5–65.0	45.0–61.0		50.9–67.0	41.8–63.2	
Non-protein nitrogen	M	13.67	15.98	1.8627	7.76	9.09	2.2240*
	S. E.	±1.33	±0.73		±0.45	±1.70	
	R	2.0–30.5	11.0–27.5		3.2–13.4	3.2–12.9	
Nitrogenous waste nitrogen	M	4.20	4.12	0.3098	2.94	3.98	2.7866**
	S. E.	±0.54	±0.24		±0.23	±0.29	
	R	1.0–10.0	2.0–6.5		1.0–6.4	1.6–8.0	
Peptode-amino nitrogen	M	9.48	11.87	1.6361	4.82	5.11	0.4563
	S. E.	±1.19	±0.72		±0.52	±0.30	
	R	1.0–25.5	6.5–21.5		0.5–12.3	0.5–9.6	

+ Water and fat – Percentage of wet weight of tissue
Free fatty acid – in milliequivalents
Glycogen and nitrogen – Percentage of dry weight (fat-free) tissue

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

Compared to that of the liver, the proximate composition of the white muscle of the infected fish does not seem to be much affected. Novertheless, a significant decrease in glycogen content ($p < 0.001$) and increase in water ($p < 0.001$) and free fatty acid content ($p < 0.01$) are discernible. While total nitrogen and protein nigrogen contents are unaffected, non-protein nitrogen content increased in the infected fish ($p < 0.05$). This is attributable to the increase in nitrogenous waste nitrogen ($p < 0.01$).

DISCUSSION

The changes in the proximate composition of the liver and white muscle of *D. hystrix* associated with the liver infection by *G. gigas* in effect conform to 'depletion' of energy reserves during starvation (see Love, 1970). The fact that the alimentary canal of the infected fish did not show any positive indication of starvation suggests that the observed depletion of fat and glycogen reserves is the direct result of disturbed hepatic function that adversely affects the synthetic and assimilative activities of the organ, whereby the food taken by the fish could not properly be utilised to replenish the energy reserves of the liver.

The observed increase in total nitrogen content of the infected fish, though at first seems to be curious, is attributable to the hypertrophy of the connective tissue of the infected liver as a result of the inflammatory reaction on the part of the host to encapsulate the invading element. This connective tissue cyst remains firmly attached to the liver tissue and it had, therefore, to be included in the biochemical analysis.

It is quite clear from the results that the biochemical composition of the white muscle of the infected fish is not affected as seriously as that of the liver. The observed heavy decrease in the glycogen content of the muscle of the infected fish seems to be a little curious. It has been shown by several workers (see Love, 1970) that during starvation, depletion of the energy reserves of an animal follows, more or less, a definite sequence, whereby the first to be depleted is glycogen followed by lipid; the protein reserves remaining unaffacted until and unless the former two components are depleted to a critical level. In fish the liver reserves are used up first, to be followed by the muscle reserves. Considering that the observed depletion of the energy reserves of *D. hystrix* infected with *G. gigas* follows the same sequence as in depletion during starvation, it is noticeable that neither the glycogen nor the lipid reserves of the liver of the infected fish decreased to a critical level to warrant mobilisation of the energy reserves of the muscle (In *D. hystrix* infected in the liver with a microsporidian, *Glugea sp.*, the fat content of the liver is found to be as low as 9.22% of the wet weight and glycogen 3.95% of the dry weight of tissue. In these fish the glycogen content of the white muscle is only slightly lower (11.10%) than that observed in the present case (Radhakrishnan and Nair, unpublished)). Possibly, the cestode infection of the liver must have caused, a general exhaustion of the affected fish, which, coupled with the disturbed hepatic function, necessitated utilisation of the rich glycogen reserves of the muscle for the normal activities of the fish.

ACKNOWLEDGEMENTS

One of us (S.R.) is thankful to the C.S.I.R., New Delhi for awarding him a fellowship during the tenure of which this work was carried out. The authors gratefully acknowledge the help received from Dr. K.S. Pillai, Reader in Chemistry, Division of Marine Chemistry, Aquarium Campus, University of Kerala by helping them in the biochemical analyses.

REFERENCES

Abrosov V.N., Bauer O.N., Bikhulov B.I. and Pavlov I.A., 1963: Further observations on ergasilosis of Coregonus peled in the lakes of Leningrad economic region. – Izv. Nauch. issled. Inst. Rech. Ryb. Khoz. **54**: 100–105.

AOAC 1945: Methods of Analyses of the Association of Official Agricultural Chemists. Procedure No. 3132. AOAC, Washington.

Kabata Z., 1958: Lernaeocera obtusa n. sp. – its biology and its effects on the haddock. – Mar. Res. **3**: 1–26.

Kosheva A.F., 1956: Effect of Ligula intestinalis and Digramma interrupta (Cestoda) on fish. (In Russian). – Zool. Zh. **35**: 1629–1632.

Lange N.A., 1967: Handbook of Chemistry. Mc Graw Hill Book Co., U.S.A.

Love R.M., 1970: The Chemical Biology of Fishes. Academic Press, London.

Mann H., 1960a: Schadwirkung des Befalls mit Ergasilus bei Schlein. – Inf. Fischwirts. **7**: 48–49.

– 1960b: Schadwirkung des parasitischen Copepoden Lernaeocera branchialis auf des Wachstum von Wittingen. – Inf. Fischwirts. **7**: 153–155.

– 1964: Vorkomen, Verbreitung und Schadwirkung von Lernaeocera minuta (T. Scott) (Copepoda parasitica). – Ver. Inst. Meeresf. Bremerhaven. **9**: 79–83.

– 1965: The significance of copepods as parasites on sea animals used economically. – Proc. Symp. Crustacea. J. mar. biol. Ass. India, Part III: 1155–1160.

Meyer-Waarden P.F. and Mann H., 1950: Beitrage zur Epidemiologie und Physiologie des parasitischen Copepoden Mytilicola intestinalis. – Arch. Fischereiwiss. **2**: 120–134.

Natarajan P. and **Nair N.B.**, 1976: Effects of infestation by Lernaeenicus 'hemirhamphi' Kirtisinghe on the biochemical composition of the host fish Hemirhamphus xanthopterus (Val.). – J. Anim Morphol. Physiol. **23**: 25–31.

Oser B.L., 1976: Hawk's Physiological Chemistry. 14th ed. TATA McGraw Hill Publ. Co. Ltd., New Delhi.

Petrushevski G.K. and **Kogteva E.P.**, 1954: Effect of parasitic diseases on condition of fish. – Zool. Zh. **33**: 395–405. (In Russian)

Petrushevski G.K. and **Shulman S.S.**, 1955: Liver nematode infections of the Baltic cod. – Tr. Akad. Nauk. Lit. SSSR., Ser. Biol. **2**: 119–124. (In Russian)

Radhakrishnan S. and **Nair N.B.**, 1980: Gymnorhynchus gigas plerocercoid (Cestoda: Gymnorhynchidae) infection of the liver of Diodon hystrix (Pisces: Diodontidae). I. Incidence and intensity of infection, histopathology and effect on hepato-somatic index. – Proc. Indian natn. Sci. Acad. **B46**: 302–309.

Reichenbach-Klinke W., Braun F., Held H. and **Reidmuller S.**, 1968: Vorläufige Ergebnisse vergleichend-physiologischer Untersuchungen an Coregonen verschiedener oberbayerischer Seen (Fettgehalt, Blutbild, Fermentspiegel, Parasitierung). – Arch. Fischereiwiss. **19**: 114–130.

Shpolyanskaya A.Yu, 1963: Changes in the chemical composition and texture of the tissue of fish with Ligula infections. (In Russian). – Dokl. mosk. sel' – Khoz. Akad. K.A. Timiryazeva. 85, 367–370.

Suryanarayanan H. and **Alexander M.**, 1972: Biochemical investigations on edible molluscs of Kerala. I. Nurtritional value of some edible bivalves. – Fish. Tech. 9: 30–36.

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III. BIOCHEMICZNE ZMIANY U ZARAŻONYCH RYB

STRESZCZENIE

Przebadano zmiany w wątrobie i białych mięśniach ryb *Diodon hystrix* zarażonych plerocerkoidami *Gymnorhynchus gigas*. Zauważono w wątrobie wydatne powiększenie zawartości wody, wolnych kwasów tłuszczowych jak też ogólnie azotu przy wydatnej obniżce zawartości tłuszczów glykogenu.

W przypadku mięśni znaczne zmiany w zawartości glykogenu jak i wzrost wody i obniżenie wolnych kwasów tłuszczowych.

Ogólnie zaistniałe zmiany można porównać do zmian występujących w czasie głodzenia.

Zaobserwowany wzrost ogólny azotu u zarażonych ryb, na pierwszy rzut oka paradoksalny, może być wytłumaczony wzrostem tkanki łącznej, powstałym w wyniku stanu zapalnego, spowodowanego na skutek osiedlenia (inkapsulacji) pasożytów.

W przypadku glykogenu zapasy w wątrobie ulegają zużyciu w pierwszym rzędzie przed mięśniowymi, stąd gdy w wątrobie stwierdza się go w ilości 3,95%, w mięśniach niewiele obniżony – 11,1%.

Wynika stąd, że w przypadku zarażenia przez *Gymnorhynchus* wątroba jest atakowana w pierwszym rzędzie bez istotnego wpływu na zdolność energetyczną żywiciela (ryby).

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ИНФЕКЦИЯ ПЕЧЕНИ Y DIODON HYSTRIX (PISCES: DIODONTIDAE ПЛЕРОЦЕРКОИДАМИ GYMNORHYNCHUS GIGAS (CESTODA: GYMNORHYNEHIDAE)

III. БИОХИМИЧЕСКИЕ ИЗМЕНЕНИЯ У ИНФИЦИРОВАННОЙ РЫБЫ

Р Е З Ю М Е

Исследовали изменения наблюдаемые в печени и белых мышцах у рыбы Diodon hystrix инфицированной плероцеркоидами Gymnorhynchus gigas. В печени наблюдалось отчётливое повышение количества воды, свободных жирных кислот и общего азота, а также уменьшение содержания жиров гликогена. В мышцах установлено значительные изменения в содержании гликогена, повышение содержания воды и уменьшение содержания свободных жирных кислот.

В целом, существующие изменения являлись близкими изменениям присутствующим во время голодания.

Установленное, на первый взгляд парадоксальное, увеличение содержания общего азота можно выяснить повышением доли соединительной ткани, вызванным воспалительным состоянием из-за заселения паразитов (инкапсуляции).

Использование резервов гликогена во первых наблюдается в печени, а затем в мышцах, отсюда в печени установлено 3,95% гликогена, а в мышцах 11,1% т.е. незначительное уменьшение содержания.

На основании полученных результатов установлено, что в случае заражения Gymnorhynchus наблюдается прежде всего атака печени, но отсутствует значимое влияние на энергетическую способность хозяина (рыбы).

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Received: 11 August 1983