

The Effect of Starvation on the Chemical Composition of Red and White Muscles in the Plaice (*Pleuronectes platessa*)

Recent studies^{1,2} have shown that red and white muscles respond differently to prolonged starvation. In the present study on the plaice (*Pleuronectes platessa*) the levels of nucleic acids have been investigated in the red and white myotomal muscles during starvation. The previous investigations³ concerning water, glycogen and protein content of the two muscles have also been extended to fish starving for up to 30 weeks.

Materials and methods. The method of capture and treatment of fish has been described previously². Fish

were killed by plunging them into liquid nitrogen (-170°C). Red muscle was dissected whilst the fish was still frozen (-20°C) from both dorsal and ventral sides. White muscle was dissected from the epaxial musculature adjacent to the dorsal fin. Frozen (-170°C) samples of dorsal and ventral red muscle were finely chopped and mixed to obtain a homogenous sample. Quick freezing the fish minimizes changes resulting from struggling or post mortem metabolism. The initial samples were taken 3 days after the fish were captured and the final sample

Table I. Changes in the concentration of red and white muscle water, glycogen and protein nitrogen

	Condition index	Water (%)		Glycogen (mg glycogen/100 g muscle)		'Insoluble' protein nitrogen (g PN/100 g muscle)		'Soluble' protein nitrogen (g PN/100 g muscle)	
		Red muscle	White muscle	Red muscle	White muscle	Red muscle	White muscle	Red muscle	White muscle
Concentration (0 weeks)	10.50	82.4 \pm 0.4 - 0.5	81.7 \pm 0.5 - 0.6	635.5 \pm 51.6	181.6 \pm 28.2	1.67 \pm 0.11	2.70 \pm 0.16	0.56 \pm 0.06	0.34 \pm 0.04
Concentration (30 weeks)	7.67	85.6 \pm 1.3 - 1.5	95.1 \pm 1.1 - 1.7	329.7 \pm 37.6	41.1 \pm 10.8	0.90 \pm 0.19	0.41 \pm 0.05	0.09 \pm 0.01	0.11 \pm 0.02
% Change	-	+	+	-	-	-	-	-	-
Significance (P)	-	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

The values given represent the means and standard errors of 8 determinations.

after 30 weeks of total starvation. The SCHMIDT THANNHAUSER procedure³ was adopted to extract RNA and DNA following pulverization of 100 mg samples of the muscle by a freeze grinding technique⁴. DNA was assayed by a modification of the Ceriotti method⁵, and RNA by the orcinol method⁶. Glucose contamination in the RNA assay was corrected for by reading at 2 wavelengths. Water contents, glycogen and nitrogen concentrations of protein fractions were determined as described previously². The significance between groups of data was calculated using the students *t*-test. In cases of unequal variance between sets the Brehrens-Fischer test was utilized⁷. Results for water content which were in a percentage form were subject to arc sine transformation before statistical analyses.

Results and discussion. The results for condition index (weight/length³ \times 1000), water content, glycogen, protein nitrogen concentration and RNA, DNA concentrations

are given in Tables I and II respectively. The changes in the concentrations of glycogen, water and proteins 'insoluble' at low ionic strength were found to be considerably more marked in the white than in the red muscle (Table I). In contrast the 'soluble' protein component of the red muscle was found to be more reduced in percentage terms than in the white muscle. The concentrations of RNA and DNA in non starved fish were found to be

¹ M. G. WALKER, J. Cons. perm. int. Explor. Mer 33, 421 (1970).

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³ G. SCHMIDT and S. J. THANNHAUSER, J. biol. Chem. 161, 83 (1945).

⁴ I. A. JOHNSTON and G. GOLDSPIK, J. Fish Biol. 5, 249 (1973).

⁵ R. W. HUBBARD, W. T. MATHEW and D. A. DUBOWIK, Analyt. Biochem. 38, 190 (1970).

⁶ N. DISCHE and K. SCHWARZ, Mikrochim. Acta 2, 13 (1937).

⁷ C. J. BLISS, Statistics in Biology (McGraw-Hill Book Company, New York 1967), vol. 1.

Table II. Changes in the concentration of red and white muscle DNA and RNA during starvation

	Wet weight of tissue ($\mu\text{g}/\text{mg}$)				Dry weight of tissue ($\mu\text{g}/\text{mg}$)			
	Red muscle		White muscle		Red muscle		White muscle	
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
Concentration (0 weeks)	0.58 \pm 0.01	2.07 \pm 0.13	0.26 \pm 0.02	1.22 \pm 0.09	3.32 \pm 0.06	11.74 \pm 0.72	1.43 \pm 0.13	6.67 \pm 0.50
Concentration (30 weeks)	0.30 \pm 0.02	0.62 \pm 0.05	0.16 \pm 0.01	0.43 \pm 0.06	2.08 \pm 0.23	4.33 \pm 0.60	4.76 \pm 1.21	11.23 \pm 1.93
% Change	-	-	-	-	-	-	+	+
Significance	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.05	< 0.05

The values given represent the means and standard errors of 8 determinations.

considerably higher in the red muscle. Expressed as wet weight of tissue the concentration of RNA and DNA were reduced by similar extents in both muscles. When expressed in terms of dry weight of tissue there was an increase in the concentration of RNA and DNA in the white muscle and a decrease in the red muscle. These changes may in part be influenced by changes in the greater rate of protein mobilisation (Table I) from the white muscle and by changes in water content. The present results are, however, consistent with changes observed in recent histological and ultrastructural studies on the effect of starvation on the red and white muscles of teleosts^{2,8}. The greater fall in the 'insoluble' protein fraction, which consists largely of myofibrillar and connective tissue proteins, in the white muscle reflects the noticeable atrophy of these fibres during starvation^{2,8}. In contrast to the white muscle little degeneration of the red muscle myofibrils was observed at the ultrastructural level in carp starving for 16 weeks⁸. The marked loss of low molecular weight proteins and decrease in RNA concentration in the red muscle probably parallels the considerable degeneration of mitochondria in this muscle during starvation⁸. The changes in RNA and DNA levels found in the plaice would seem to be correlated with the observed loss of euchromatin material from the nuclei of both red and white fibres of the carp⁸, but whether this alone could account for the reductions is uncertain.

The present study indicates that the white muscle contractile proteins are preferentially utilized by the fish during starvation. There would, therefore, appear to be a differential response by the two principle muscle types of teleosts to inanition. It seems possible that severe changes in the nutritional state of a fish might influence the divi-

sion of labour between the myotomal muscles. Such temporal changes are already thought to occur with respect to seasonal changes and migrations^{9,10}.

Zusammenfassung. Die Wirkung von experimentellem Hunger auf rote und weisse Muskeln der Scholle, *Pleuronectes platessa*, wurde untersucht. In roten wie in weissen Muskeln wurde eine starke Verminderung von Protein und Glycogen gefunden, während der RNS- und DNS-Gehalt in beiden Muskeln herabgesetzt war.

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⁸ S. PATTERSON and G. GOLDSPIK, *Z. Zellforsch.* 116, 375 (1973).

⁹ S. L. BOSTROM and R. G. JOHANSSON, *Comp. Biochem. Physiol.* 42 B, 533 (1972).

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