SUMMARY

1. A rapid procedure for large scale preparation of mitochondria is presented.

2. Some biochemical properties of mitochondrial powders have been studied. It was found that these preparations, or modifications thereof, oxidized a number of substrates.

3. Measurements of oxidative phosphorylation, adenosine triphosphatase, and ATP–P_i exchange activities have been carried out with these preparations.

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Received December 12th, 1957

ELASTOIDIN: A TWO-COMPONENT MEMBER OF THE COLLAGEN CLASS*

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Elastoidin, the large translucent fiber of the fins of selachian fish, is a member of the collagen class, as evidenced by its characteristic wide-angle X-ray diffraction pattern[1,2], the presence of a 600–800 Å period in the fibril[3,4], and its amino-acid distribution[4,5]. One unusual feature of its composition, however, is the presence of more than 6% tyrosine[4,5]. This amino acid rarely accounts for more than 1% of purified vertebrate collagen[6]. The fibers have a relatively high shrinkage temperature, 60–64°, and regain most of their length on cooling[4,5]. Elastoidin has been said to produce no gelatin on boiling, although it has been solubilized to the extent of 98% by autoclaving seven times at 120° [5].

* This is publication No. 221 of the Robert W. Lovett Memorial for the Study of Crippling Disease, Harvard Medical School at the Massachusetts General Hospital, Fruit Street, Boston. This study was aided by grant No. A 90 (C 6–7) from the National Institute of Arthritis and Metabolic Diseases of the United States Public Health Service.

** This work was done during the tenure of an Established Investigatorship of the American Heart Association.

References p. 270.

This note reports the separation of elastoidin into two components, one of which contains most of the tyrosine in high concentration and the other a gelatin with comparatively low tyrosine content and most of the glycine, proline and hydroxyproline.

MATERIALS AND PROCEDURES

Elastoidin fibers from the fins of *Carcharias glaucus* (two different samples), *Mustelus vulgaris* and *Squalus acanthias*, cleaned with trypsin, were obtained through the kindness of Prof. E. FAURÉ-FREMIET. A fresh sample from *Squalus* was obtained from the Marine Biological Laboratories, Woods Hole, Mass. and cleaned with trypsin. These were ground in the Wiley Mill or cut up with scissors. A portion was analyzed without further treatment and other samples were autoclaved in distilled water at 15 lb. pressure for 16 h. A brownish residue was separated by centrifugation and washed twice in small volumes of boiling water, the wash water being added to the supernatant. Both residues and solutions were dialyzed against water and lyophilized.

These were analyzed for hydroxyproline[6,7], proline[8], glycine[9], and tyrosine[10]. Hydrolysis was accomplished in 6 *N* HCl in a sealed tube in an oil bath at 138° for 3 h. The first three amino acids were measured in the same hydrolysate. Tyrosine was measured colorimetrically in an alkaline hydrolysate[10]. In addition an alkaline solution of residue from *C. glaucus* fibers dissolved by heating in 0.1 *N* NaOH at 80–90° for a few min was analyzed for tyrosine by the spectrophotometric method of BENCZE AND SCHMID[11].

RESULTS

The percent of water-soluble and insoluble fractions and their respective contents of glycine, hydroxyproline, proline and tyrosine are reported in Table I. Two different preparations of *C. glaucus* and *S. acanthias* were analyzed. A tyrosine content of 25 % determined from the U.V. absorption spectrum in 0.1 *N* NaOH[11] of the residue from sample 1 of *C. glaucus* agreed well with that measured by the colorimetric method.

Losses of substance ranging from 6 to 11 % occurred during preparation.

It is curious that the gelatins obtained from one of the two samples of elastoidin

TABLE I

ANALYSIS OF WHOLE FIBER AND THE WATER-SOLUBLE (GELATIN) AND WATER-INSOLUBLE (RESIDUE) FRACTIONS

g/100 g dry weight

		Carcharias glaucus			*Mustelus vulgaris*			*Squalus acanthias*		
		Fiber	*Gelatin*	*Residue*	*Fiber*	*Gelatin*	*Residue*	*Fiber*	*Gelatin*	*Residue*
Percent of whole fiber	(1)	100	77.8	10.7				100	85.2	22.2
	(2)	100	68.7	25.8	100	74.6	14.3	100	75.9	14.5
Glycine	(1)	19.7	25.7	—				20.9	24.0	12.3
	(2)	21.7	25.0	14.1	22.8	27.8	13.1	21.2	23.6	10.2
Hydroxyproline	(1)	8.1	11.0	—				5.0	5.8	2.3
	(2)	7.5	9.0	3.4	6.3	9.0	2.7	5.6	6.0	2.3
Proline	(1)	10.7	12.0	—				13.2	14.8	7.9
	(2)	11.3	10.1	7.3	11.0	12.2	7.4	13.4	10.6	7.2
Tyrosine	(1)	6.4	1.95	25.9				8.9	5.3	25.3
	(2)	7.5	2.9	18.0	6.7	3.8	20.5	7.5	3.6	22.1

Nos. 1 and 2 indicate separate batches of fibers. No. 1 of *C. glaucus* was a much larger fiber than No. 2. No. 1 of *S. acanthias* was obtained fresh.

References p. 270.

of *C. glaucus* and *S. acanthias* contain somewhat less proline (average of three determinations in close agreement) than did the whole fibers.

DISCUSSION

It would appear that elastoidin consists of a tightly bound mixture of a characteristic collagen which yields a water-soluble gelatin on autoclaving, and a water-insoluble residue containing a remarkable amount of tyrosine, 18–25 %, and relatively little hydroxyproline. This non-collagenous component accounts for 14–26 % of the total fiber. Apparently, repeated autoclaving will dissolve this protein[5].

Sugar analyses, to be reported in detail elsewhere, of the whole fiber of *C. glaucus* revealed relatively little carbohydrate, 0.7 % hexose, 0.03 % hexosamine, and 0.04 % uronic acid. Most of this remained with the gelatin fraction.

Histological studies of transverse sections of elastoidin fibers reveal a series of concentric layers. Electron microscopy of fragmented preparations show only cross-striated ribbons[4] (GROSS, unpublished). It is possible that the insoluble fraction is not sufficiently dispersed by the usual fragmentation procedures to appear in appreciable amounts in electron micrographs of such preparations. It is interesting that evidence of this component in the X-ray diffraction diagrams has not been reported.

Incubation of *C. glaucus* fibers for 48 h with collagenase (*Cl. histolyticum*), trypsin, chymotrypsin, pepsin, hyaluronidase, 0.1 *N* NaOH and 0.1 *N* HCl did not separate the two fractions. The amounts of collagen dissolved by the agents listed in the above order amounted to 5 %, 12 %, 20 %, 70 %, 0.0 %, 0.0 % and 0.0 % of the original fiber, as measured by hydroxyproline in the supernatant fluid. Pepsin dissolved nearly all the tyrosine-containing component as well as the collagen. A more complete description will be reported elsewhere.

SUMMARY

Elastoidin, a member of the collagen class of proteins, proves to be a tightly bonded mixture of a characteristic collagen which may be extracted by autoclaving as a water-soluble gelatin, and a water-insoluble, tyrosine-rich residue.

The cleaned fibers of three species of selachian fishes yielded 14 to 26 % of the insoluble residue, containing 18 to 25 % tyrosine.

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Received November 11th, 1957