two cannulas was about 40 mm. The dye made it possible to observe the peristaltic wave of contraction more clearly and to take motion pictures of the process. The current necessary to stimulate the preparations was passed through the lumen of one of the cannulas. Figure 3 shows the time course of pressure change at two ends of the beat as the right end of the heart is stimulated. Two thinner cannulas connected directly to pressure transducers were advanced through the lumen of the wider cannula until the opening was extended about 3 mm into the heart. The illustrated pressure traces correspond to a clearly propagated wave of contraction. The pressure at the stimulated right end of the heart (P_r) increased transiently for about 0.5 sec while the pressure at the left end (P_l) continued to increase until the contractile wave reached the unstimulated end. Notice also that there is a few hundred msec delay between the initiation of the contraction of the left end and the first indication of contraction at the right end. These observations may be explained on the basis of the propagation of two pressure waves: a pressure wave which is passively propagated, and a dynamic pressure wave which travels with the speed of the electrical excitation (1-2 cm/sec).

Conclusions: Experiments of the type illustrated in Figures 1 and 2 indicate that a 10% change sarcomere length or total length are completed within about 50 msec. This time interval is short compared to both the rising and falling phase of the twitch suggesting that the time course of the twitch tension corresponds to changes in degree of activation of the myofilaments. Figure 2 further suggests that a first approximation of the developed wall tension may be obtained considering only the present length and the time since stimulation (i.e., disregarding the preceding changes in length). A computer simulation along these lines is presently being attempted and the results are being compared to the results obtained with the intact heart (Figure 3). Figure 3 shows that the heart can develop a pressure difference between its two ends. In this respect the computer simulation may help to elucidate how factors such as viscosity, speed of propagation and diastolic pressure determine the efficiency of the valveless tunicate heart.

HISTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERISTICS OF RED AND WHITE MUSCLE OF THE SPINY DOGFISH, *Squalus acanthias*

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The spiny dogfish, *Squalus acanthias*, like many other poikilotherms is able to survive for many weeks without food. During this period it must rely on endogenous stores. Fuel utilization has been poorly studied in fish but a high degree of dependence on lipid metabolism during starvation is to be expected. This study examines the possibility that lipid may be a significant fuel for skeletal muscle of starving fish.

To begin analysis of the specific properties of shark muscle that might be correlated with its biochemical and physiologic properties, a morphologic survey was undertaken utilizing standard methods. Portions of both red and white striated muscle were preserved in a fixative containing 2 parts 4% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2), 1 part 8% paraformaldehyde and 1 part elasmobranch saline, and other pieces were snap-frozen and maintained in the frozen state at -30°C. Tissue for ultrastructure examination was embedded in Epon 812 and semi-thin sections were stained with Toluidine blue and examined in the light microscope for selection of areas to be thin-sectioned. Frozen sections cut at 6 μ were stained for lactic (LDH) and succinic dehydrogenase (SDH) activity by the azo dye method using a chromogenic substrate, and magnesium ATPase (ATP-ase) activity was demonstrated by a standard method (Brooke and Kaiser, Arch. Neurol. 23:369, 1970).

Hematoxylin and eosin staining revealed differences between the red and white muscle. The red muscle has loose myofiber texture with clusters of mitochondria and lipid granules lying between the individual myofibrils. Transverse semi-thin sections stained with Toluidine blue have an ordered reticular appearance due to a pattern of rosettes resulting from adjacent rings of myofibrils surrounding cores of mitochondria and lipid droplets. Large quantities of osmium-stained lipid droplets lay beneath the sarcolemma. The white muscle is more compact, without the rosette appearance in transverse sections; histologically, it resembles striated muscles in other vertebrate species although numerous lipid droplets are also present.

When stained to demonstrate dehydrogenase activity, a uniform pattern of LDH- and SDH-containing fibers were demonstrated in white muscle which did not vary significantly in intensity from similar activity demonstrated within the red muscle. However, myofibers in red muscle demonstrated grouping of myofibers reminiscent of the "checkerboard" pattern present in higher vertebrates including man. Both white and red muscles contained magnesium ATPase activity with the more intense reaction present in the white muscle. Table 1 illustrates relative staining intensity after 30 minutes of incubation in the histochemical reagents on a scale of 0-4+.

TABLE 1

Histochemical assay of succinic dehydrogenase (SDH), lactate dehydrogenase (LDH) and magnesium ATP-ase (ATP-ase) in skeletal muscle of the spiny dogfish

	Stain	Reaction
White muscle	LDH	+++
	ATPase	+++
	SDH	++
Red muscle	LDH	+++
	ATPase	++
	SDH	+++

"Reaction" refers to visual intensity of staining on a scale of 0 to 4, using comparable conditions of 30 min. for LDH and SDH staining and 5 to 10 min. for ATP-ase activity.

The ultrastructure of the red muscle confirmed the presence of large numbers of mitochondria and apparent neutral fat droplets lying between each myofibril within the myofibers. In typical areas, the myofibrils measure 1.5 μ in diameter and are separated from adjacent myofibrils by a space of 3-4 μ filled with masses of mitochondria ranging in size from 0.6 -1.0 x 3.6-4.5 μ in greatest dimensions. Interspersed and intimately related to the mitochondria are large numbers of lipid droplets that are composed of homogeneous osmophilic, fine granular material which have no peripheral membrane. The mitochondria are densely packed with many tubular and few linear cristae, a finding which may be interpreted as indicative of high oxidative capacity. Scattered matrical dense granules are present, measuring about 300-400 Å. Occastional lead-stained granule aggregates consistent with glycogen lie between individual myofilaments within the myofibrils and small numbers of these granules are present amongst the mitochondria between the myofibrils.

The white muscle has a more conventional structure with closely packed myofibrils with only occasional scattered mitochondria and lipid droplets lying between them. Both red and white muscle have relatively little glycogen stores as demonstrated by lead staining of granular aggregates in the ultrastructure.

The special physiology of these muscles may be related to a biochemical system that stores energy as lipid droplets and utilizes them rather than glycogen, for oxidative metabolism. The large number and high density of mitochondrial cristae in the mitochondria is consistent with this notion, as is their intimate relationship to the lipid droplets. Supported by NSF Grant PCM75-14322A02 and NIH Grant IR01 AGO 0961-01.

ROLE OF METABOLISM AND TRANSPORT IN THE EXCRETION OF PHENYLACETIC ACID AND 2,4-DICHLOROPHENOXY ACETIC ACID BY MARINE FISH.

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Phenylacetic acid (PA) has been widely utilized to examine species differences in the enzymatic pathways responsible for detoxification of many chemicals via conjugation with amino acids. Using PA and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) to characterize these pathways in marine fish, James et al. (Bull. MDIBL 13:59-62, 1973, Xenobiotica 7:393-398, 1976) demonstrated that the taurine conjugates (PAT and 2,4-DT) were the major metabolites produced by both winter flounder and dogfish. These compounds were excreted almost exclusively via the urine. However, these and subsequent studies (Pritchard and James, J. Pharmacol. Expt. Therap., in press; James, unpublished) demonstrated an inverse relationship between the extent of metabolism (i.e., taurine conjugation) and the rate of excretion. Since conjugation is generally held to produce more readily excreted metabolites, this was a surprising observation. Therefore, using the winter flounder, *Pseudopleuronectes americanus*, and the spiny dogfish, *Squalus acanthias*, we attempted to answer the following questions: (1) Were parent compound and taurine conjugate transported at substantially different rates? (2) Did differences in renal transport underlie differences in excretion within a single species, e.g., 2,4-D (rapid) vs. PA (slow) in the winter flounder, or between species, e.g., 2,4-D in the flounder (rapid) vs. the dogfish (slow)?

Techniques utilized were the isolated flounder tubule preparation of Forster (Science 108:65-67, 1948) and renal clearance studies in both flounder and dogfish. The details of both flounder preparations have been previously described (Am. J. Physiol. 233:F126-132, 1977). The dogfish clearances were performed as described by Guarino and Anderson (Xenobiotica 7:143, 1976).

Table 1. Concentration dependence of the uptake of phenylacetic acid (PA) and its taurine conjugate (PAT) by isolated flounder tubules.*

[ ] μMolar	1	10	100	1000
PA (5)	8.7 ± 1.1	8.0 ± 0.9	5.6 ± 0.7	3.6 ± 0.3
PAT (4)	9.9 ± 1.6	9.8 ± 1.7	8.9 ± 1.3**	5.8 ± 0.5**

* Uptake expressed as the mean tissue-to-medium ratio ± S.E. The figure in parentheses is the number of animals tested. Tubules were incubated for 60 min. at 15°C under 100% O_2.

** $p < 0.05$ versus PA at the same concentration

We began by comparing the transport of PA and PAT *in vitro* and *in vivo*. As shown in Table 1, isolated flounder tubules accumulated both PA and PAT to concentrations much greater than that in the bathing medium. Uptake was concentration dependent. Uptake of both compounds also required metabolic energy and was sensitive to probenecid (Figure 1). Since cyanide (1 mM) reduced the tissue-to-medium ratio (T/M) to essentially 1 for both compounds, significant intracellular binding was absent. Thus, uptake of PA and PAT behaved as if it were mediated by the organic anion transport system and little difference was

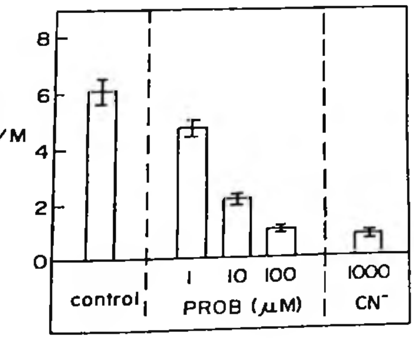

Figure 1. Effect of probenecid and cyanide on transport of phenacetyltaurine (PAT) by flounder renal tubules *in vitro*. The teased tubules were incubated with 1 μM ^{14}C - PAT in Forster's saline for 60 min. at 15°C under 100% O_2. Results are expressed as the mean tissue-to-medium ratio (T/M) ± SE (bars) for determinations in five animals. * = $p < 0.05$ vs. the control.