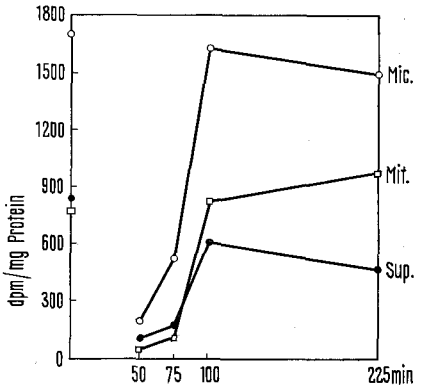

Fig. 3. Effect of a single injection of puromycin on leucine-^{14}C incorporation into microsomal, mitochondrial, and supernatant fractions of mouse liver. Puromycin was injected at zero time. Leucine-^{14}C was given 45 min prior to the time interval indicated. Each value is an average of at least 3 mice, with the exception of the 75 and 225 min values each of which are an average of 2 mice. The control values on the ordinate represent an average of 15 mice.

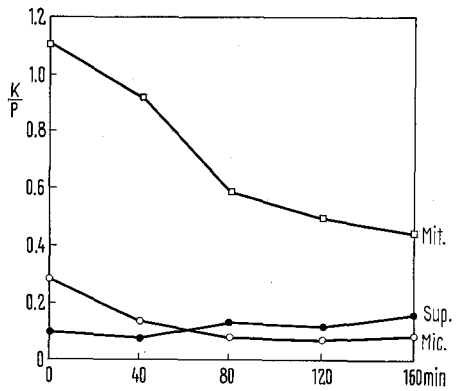

Fig. 4. The effect of multiple injections on catalase activity. Puromycin was injected at zero time, and at 40 min intervals. Values at 40 min represents one injection; 80 min, 2 injections; etc. The 40 min value is an average of 7 mice, and the other values are an average of 2 mice. The control values are an average of 15 mice.

different batches of puromycin as reported by STUDZINSKI and BASERGA[15]. We used only that puromycin which effected maximum inhibition.

Multiple injections of puromycin, given in the same schedule as shown in Figure 4, maintained inhibition of leucine incorporation through 160 min. Under these conditions, multiple injections of puromycin depressed the catalase activity of the liver mitochondrial fraction more than 50% of normal (Figure 4). As protein synthesis was inhibited under these conditions, as judged by the inhibition of leucine incorporation, the results indicate that depressed catalase activity was due to inhibition of the synthesis of at least the protein portion of the catalase molecule. If the catalase was assembled from pre-formed parts, puromycin would presumably not be inhibitory, especially at the early time interval. On this basis, the results are in accord with the concept[6,16] and evidence[16] that the catalase protein is formed in the microsomes and is transported to the organels of the mitochondrial fraction. Also, the early inhibitory effect of puromycin on catalase activity is indicative of the rapid turnover of the catalase molecule. Incubation in vitro of puromycin with the mitochondrial fraction had no effect on catalase activity[17].

Zusammenfassung. Es wurde die Dauer der Hemmung der Leucin-^{14}C-Inkorporation in Proteine von subzellulären Fraktionen der Mäuseleber nach einmaliger und mehrfacher Gabe von Puromycin untersucht, ebenso der Einfluss von Puromycin auf die Katalaseaktivität der Leber.

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Biochemical Changes of Muscle Proteins in Goldfish (*Carassius auratus*) During Thermal Acclimatization

DAS and PROSSER[1] demonstrated a translational compensation of protein synthesis in goldfish skeletal muscle during thermal acclimatization. The accelerated incorporation of ^{14}C-leucine into proteins of subcellular fractions from the 5 °C-adapted goldfish muscle over the 25 °C-adapted fish tissue was shown by DAS[2] to be relatively uniform, but this augmentation was greater in 'microsomal' than in 'nuclear', 'mitochondrial' or 'soluble' fraction. However, the increase of the total protein content during cold adaptation was much less in muscle as compared with both liver and gill of this fish[2]. The present investigation was aimed at comparing the yields of contractile, sarcoplasmic and collagenous protein fractions and the levels of radio-active amino acid incorporation into these fractions from the skeletal muscle of cold- and warm-acclimatized goldfish, *Carassius auratus*.

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Table I. Amino acid incorporation into different fractions of muscle protein in goldfish adapted to high and low temperatures

Temperature of acclimatization	Collagenous proteins	Sarcoplasmic proteins	Myosin protein	Actin protein
5 °C	164.90 ± 79.04	247.30 ± 31.73	167.50 ± 74.38	114.00 ± 12.84
25 °C	15.87 ± 8.15	25.59 ± 9.88	17.90 ± 7.68	10.50 ± 5.82
Significance: (*t*-test)	5° > 25° ($P = 0.01$)	5° > 25° ($P = 0.001$)	5° > 25° ($P = 0.01$)	5° > 25° ($P = 0.001$)

DPM ^{14}C-leucine per mg protein residue per 12 h at 5 °C (mean of 5 samples ± S.D.).

Goldfish, adapted to 5° and 25 °C for more than a month, were administered with ^{14}C-leucine i.p. at the dose of 2 μc/0.1 ml saline/10 gm fish and kept in 5 °C for 12 h[1] before they were sacrificed. Excised and weighed trunk muscles from these fish were used for the separation of myosin, actin, sarcoplasmic and collagenous protein fractions according to a method similar to that described by BĀRĀNY et al.[3]. The entire procedure was conducted at 5 °C. The 4 protein fractions were then treated with cold perchloric acid (5% in the final volume) and from the precipitated macromolecules dried protein residues were obtained and the ratio-activity counted as described earlier by DAS and PROSSER[1]. The levels of ^{14}C-leucine incorporation into the 4 protein fractions and the extractibility of these fractions from the muscle of 5 °C- and 25 °C-adapted goldfish were recorded as in the Tables I and II.

Table II. Extractibility of different protein fractions from muscle of goldfish adapted to high and low temperatures

Temperature of acclimatization	Collagenous proteins	Sarcoplasmic proteins	Myosin protein	Actin protein
5 °C	93.1 ± 6.9	39.5 ± 5.0	24.8 ± 1.6	8.6 ± 1.5
25 °C	95.6 ± 7.9	30.8 ± 6.8	19.7 ± 1.5	9.8 ± 1.7
Significance: (*t*-test)	5° = 25° ($P = 0.5$)	5° > 25° ($P = 0.05$)	5° > 25° ($P = 0.001$)	5° = 25° ($P = 0.2$)

mg protein/g wet wt. of muscle (mean of 6 samples + S.D.)

The results shown in Table I reveal a uniform 9- to 10-fold augmentation of radio-active leucine incorporation into all the protein fractions of the cold-acclimatized fish muscle over the warm-acclimatized fish tissue, which is similar to a 7-fold increase in this incorporation into total protein due to cold adaptation recorded by DAS and PROSSER[1]. Although the enhancement of protein synthesis in skeletal muscle of this fish during adaptation to low temperature was found to be not completely non-specific in relation to the subcellular components[2], yet for the contractile, collagenous and sarcoplasmic proteins this seems to be a general and uniform phenomenon. In both the thermal categories of fish, the highest level of radio-active counts was obtained in the sarcoplasmic protein fraction similar to the observation of DAS[2] regarding the distribution of the maximal counts between 'microsomal' and 'mitochondrial' fractions.

As presented in Table II, the extractibility of the protein fractions from muscle in both the thermal groups of fish exhibit the following order: collagenous > sarcoplasmic > myosin > actin. The yields of only sarcoplasmic and myosin proteins demonstrate respectively 29% and 26% increase in cold-acclimatized goldfish muscle over warm-acclimatized fish tissue and this is less than the 59% augmentation of total protein content (mg/100 ml protein) shown by DAS[2]. Hence, the total protein and even the protein fractions exhibit little accumulation in spite of the accelerated level of synthesis of proteins in the skeletal muscle of goldfish during cold-acclimatization. This is in contrast to the remarkable increase of the protein content of both liver and gill in this fish due to cold-adaptation[2]. Nevertheless, a specific compensatory augmentation in the steady state concentrations of the myosin and sarcoplasmic proteins plausibly increase the contractile and bio-energetic potentialities of the skeletal muscle in goldfish during acclimatization to low temperature[4].

Zusammenfassung. Kälteadaptierte Goldfische (*Carassius auratus*) bauen mehr Leuzin in Muskelproteine ein, wobei die Steigerung in verschiedenen Muskeleiweissfraktionen gleich ist. Aus kälteadaptierten Goldfischmuskeln ist mehr Myosin und sarkoplasmisches Eiweiss extrahierbar.

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