The Duration of Action of Single and Multiple Injections of Puromycin on Leucine Incorporation and Hepatic Catalase Activity

Puromycin is an inhibitor of protein synthesis and has been used in in vitro and in vivo studies of protein synthesis and enzyme induction [1–4]. The purpose of the present studies was to: (a) determine the duration of the inhibitory effect of single and multiple injections of puromycin on the incorporation of leucine-^{14}C into proteins of subcellular fractions of mouse liver, and (b) determine the effect of puromycin on hepatic catalase activity.

Materials and methods. The average weight of the Strong A male mice used in this study was 33 g. L-leucine-U-^{14}C (New England Nuclear, 200 mc/m*M*) was neutralized, and 3.3 µc per 0.2 ml injected i.p. Puromycin dihydrochloride (Nutritional Biochemicals Corporation) was dissolved in isotonic saline containing 0.04 *M* phosphate buffer, pH 7.2, to make a 0.5% solution. The puromycin (2.5 mg) was given i.p. (Injection of 5 mg of puromycin gave erratic results and sometimes killed the mice.) The mice were killed by cervical separation, and the livers fractionated by the method of SCHNEIDER and HOGEBOOM [5]. The trichloroacetic acid (TCA) insoluble material of the various fractions was washed twice with 5% TCA and once with methanol. The methanol was about 8 °C. The precipitated protein of the various fractions was solubilized with Hyamine (Packard Instrument Co.), and counted in a Packard Tri-Carb liquid scintillation spectrophotometer. Catalase activity is expressed in terms of the first order velocity constant divided by the mg protein (K/P) [6]. Protein was determined by the method of LOWRY [7] as modified by HENRY [8].

Results and discussion. Leucine was chosen for these experiments because leucine participates in relatively few metabolic reactions other than protein synthesis and is incorporated into proteins largely as leucine [9–12] by de novo synthesis of protein [13]. It has also been shown that most proteins differ relatively little in their leucine content [14].

The first experiment (Figure 1) shows that maximum incorporation of leucine-^{14}C into subcellular fractions of mouse liver is observed at 45 min after injection. Sufficient leucine-^{14}C is available to maintain high incorporation levels for 180 min. A single injection of 2.5 mg of puromycin effectively inhibits incorporation of leucine-^{14}C into all subcellular fractions by 10 min (Figure 2). The duration of maximal inhibition was approximately 45 min for all fractions. After 45 min, there was a rapid rise in all subcellular fractions. The low plateau values in Figure 2 can be ascribed to excretion, breakdown, or dilution of the labeled leucine during the period of maximum inhibition of leucine-^{14}C incorporation by puromycin (compare with Figure 3). The following experiment (Figure 3) was designed so that an adequate supply of leucine-^{14}C was available for incorporation. Puromycin was injected at zero time. Mice were killed at 50, 75, 100, and 225 min. However, leucine-^{14}C was given 45 min before the mice were killed. The results in Figure 3, assessed with the results in Figure 2, show that following maximal inhibition of leucine-^{14}C incorporation, a return to control levels of incorporation was obtained by 100 min in the presence of sufficient leucine-^{14}C. Few or no counts were observed in the acid-soluble fraction nor in hot TCA washes. Experimental control, that is the addition of radioactive leucine to the homogenate immediately before beginning the fractionation and followed through in the same manner as the experimental, showed no counts in any of the fractions. Parenthetically, mention should be made that we, too, observed differences in potency for

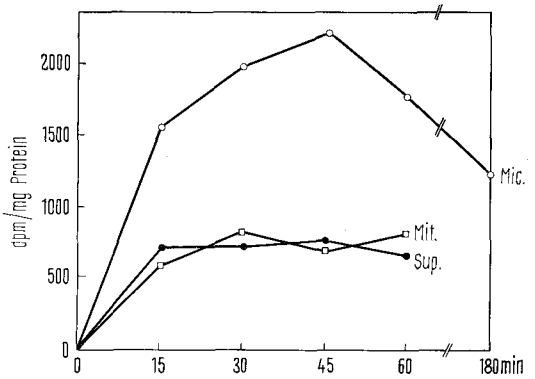

Fig. 1. Leucine-^{14}C incorporation into microsomal (Mic), mitochondrial (Mit), and supernatant (Sup) fractions of mouse liver. Leucine was injected at zero time. Each value is an average of at least 4 mice.

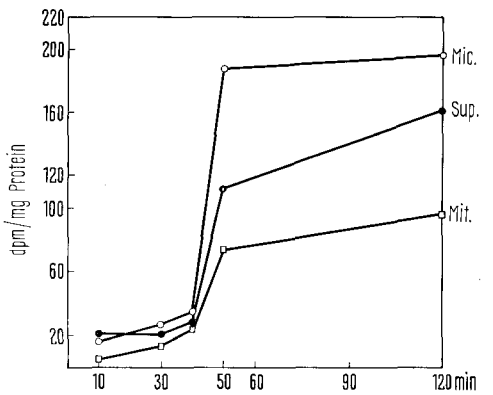

Fig. 2. Effect of a single injection of puromycin on leucine-^{14}C incorporation into microsomal, mitochondrial, and supernatant fractions of mouse liver. Puromycin was injected at zero time, and leucine-^{14}C 5 min later. Each value is an average of at least 3 mice, with the exception of the 120 min value which is an average of 2 mice. Control values are shown in Figure 3.

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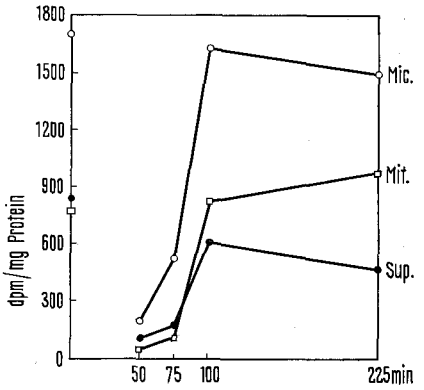

Fig. 3. Effect of a single injection of puromycin on leucine-^{14}C incorporation into microsomal, mitochondrial, and supernatant fractions of mouse liver. Puromycin was injected at zero time. Leucine-^{14}C was given 45 min prior to the time interval indicated. Each value is an average of at least 3 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.

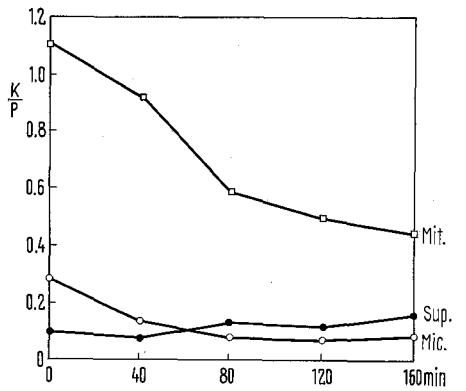

Fig. 4. The effect of multiple injections on catalase activity. Puromycin was injected at zero time, and at 40 min intervals. Values at 40 min represents one injection; 80 min, 2 injections; etc. The 40 min value is an average of 7 mice, and the other values are an average of 2 mice. The control values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