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Increased Turnover of Mitochondrial Constituents in Cardiac Hypertrophy
and Acute Hypoxia in the Rat²

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MASTER

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Under normal conditions, cardiac muscle derives its energy almost exclusively from oxidative metabolism localized in mitochondria. Since the mitochondrion is the principle site of myocardial ATP synthesis, changes in mitochondrial functional mass may have important consequences to the metabolism of the rest of the cell. We now know that mitochondria are semiautonomous organelles which have independent systems for the synthesis of nucleic acids and protein (1-4). Heart mitochondrial components turn over rapidly with a half-life of about 5-6 days (5), indicating a capacity for rapid synthesis. In these experiments we sought to study the effects of experimentally-produced cardiac hypertrophy, and of severe hypoxia on the synthesis and turnover of mitochondria, and of other cardiac muscle components. Mitochondria are particularly suitable for these studies since they are predominantly (over 95%) localized in the cardiac muscle cell in whose function we are most interested. Furthermore, as the tissue receptor for oxygen, mitochondria may be particularly sensitive to changes in tissue oxygen tension or to levels of oxygen consumption.

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Experimental cardiac hypertrophy was produced by banding the ascending aorta in rats (6). As seen in Fig. 1 a 20-40% increase in heart weight is achieved within 36-48 hours after aortic constriction.

Changes in mitochondrial mass after aortic constriction were estimated from the activity of mitochondrial respiratory enzymes in homogenate and mitochondria. As seen in Fig. 2, mitochondrial mass appears to increase proportionally greater than total myocardial proteins one day after aortic banding as indicated by the increase in homogenate specific activities of cytochrome oxidase, succinate cytochrome c reductase, and succinic dehydrogenase. Total cardiac activities of these enzymes increase to a greater extent than does total cardiac protein content. Mitochondrial enzymes therefore appear to be synthesized more rapidly than other protein components in early hypertrophy. This preferential synthesis of mitochondrial proteins is less apparent three days after banding.

Preferential synthesis of mitochondrial proteins in early cardiac hypertrophy is also indicated by the relative incorporation of ^3H -leucine into various myocardial subcellular fractions. Table I shows that 24 hours after aortic banding the increment in incorporation of ^3H -leucine into mitochondrial proteins was greater than that into myofibrillar or supernatant proteins. It may be concluded that mitochondrial proteins are rapidly synthesized during the acute phase of cardiac hypertrophy.

To gain insight into the dynamics of synthesis and degradation of mitochondrial proteins, we studied the effects of two experimental procedures: (1) cardiac hypertrophy produced by aortic constriction; and (2) exposure to severe hypoxia, on the turnover of mitochondrial proteins. We will first discuss the effect of hypoxia and will later return to cardiac hypertrophy.

The rationale of the experiments to be described was to label mitochondrial protein, or a specific mitochondrial cytochrome several days before the experimental intervention. The effects of the experimental procedure on the specific radioactivity of that component is then measured. A decrease in specific radioactivity compared to controls reflects increased synthesis of new unlabeled material. Changes in the rate of destruction are measured by changes in the total cardiac radioactivity of that component.

In the experiment shown in Fig. 3, mitochondrial proteins were pre-labeled with ^{14}C -guanido-arginine. The rapid normal turnover of mitochondrial proteins is apparent. After exposure of rats to a six-hour period of 4-5% hypoxia, the specific activity of mitochondrial proteins was 15-20% less than controls. This decrease is indicative of synthesis of new unlabeled mitochondrial proteins, presumably secondary to increased destruction produced by hypoxia. The slope of the decay curves for hypoxic and control animals are substantially the same, indicating that mitochondria which escape destruction during the hypoxic period are not more susceptible to destruction later.

These conclusions are confirmed by experiments using cytochrome c labeled with ^3H - δ -aminolevulinic acid which is a specific heme precursor and is a non-reutilizable precursor. Accurate measurements of total cytochrome c content can also be made which facilitate interpretation of the results.

Cytochrome c is known to be bound to the inner mitochondrial membrane, and to turn over at the same rate as the particulate cytochromes b, and aa₃ (5 7).

The specific activity of cytochrome c (Fig. 4) and also of isolated purified heme aa₃ decreased by 15-30% after exposure to low oxygen atmosphere. No dilution was present at the end of the six-hour hypoxic period, but it was present 6-12 hours after return to a normal atmosphere. This indicates that

mitochondria may be destroyed during hypoxia, but they are resynthesized only after return to normal oxygen atmosphere. Also seen in Fig. 4 is that hypoxia causes no decrease in myosin specific radioactivity. This indicates that mitochondria are more sensitive to the effects of hypoxia than is myosin.

Hypoxia leads to a significant decrease in cytochrome c content by the end of the six-hour hypoxia period (Fig. 5.) The level returns to normal six hours later indicating a brisk resynthesis. Thus it seems clear that hypoxia results in a destruction of mitochondrial components which are rapidly synthesized after termination of the hypoxia.

That mitochondrial resynthesis occurs predominantly after return to normal oxygen tension is confirmed by experiments measuring the incorporation of ^3H -leucine into mitochondrial protein during and after hypoxia. Incorporation was at control levels during hypoxia, but increased 200-300% two to three hours after return to normal oxygen tensions (Fig. 6). The level of incorporation returns to normal by 24 hours.

The effect of aortic constriction in cytochrome c turnover was also studied. As seen in Fig. 7, cytochrome c content increases by about 10% after aortic banding. After correction is made for the increase in cytochrome c content a 15-20% dilution of cytochrome c specific activity is present. The decrease in specific radioactivity, and in total cardiac cytochrome c radioactivity compared to controls, indicates an increased turnover immediately after banding. The observed decrease in corrected specific radioactivity was unchanged three days after banding, indicating that the rate of destruction had returned toward normal.

From these data, and from the turnover rate of cytochrome c, estimations of the rate of destruction and synthesis of cytochrome c can be made. It

appears that rate of destruction has increased considerably during the first 24 hours after banding. This increased rate of destruction is compensated by a three-fold increase in synthesis rate. Three days after banding the rate of destruction and synthesis have returned toward normal.

Summary

1. Twenty-four hours after production of cardiac hypertrophy mitochondrial mass increases proportionately greater than other cardiac muscle proteins.
2. There is an increased rate of mitochondrial destruction 24 hours after aortic banding which returns toward normal thereafter.
3. Severe hypoxia leads to mitochondrial destruction.
4. Mitochondrial components are rapidly resynthesized after return to a normal oxygen tension.

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Figure Legends

- Fig. 1. Changes in heart weight after banding the ascending aorta of rats.
From Nair et al. (6).
- Fig. 2. Effect of banding the ascending aorta of rats on the cardiac respiratory enzyme activities. From Rabinowitz et al. (8).
- Fig. 3. Effect of a 6-hour period of 4-5% oxygen on the specific activities of mitochondrial protein previously labeled with ^{14}C -guanido-arginine.
From Rabinowitz et al. (8), Aschenbrenner, Zak and Rabinowitz (9).
- Fig. 4. Effect of a 6-hour period of 4-5% O_2 on the specific activities of cytochromes c, and heme a prelabeled with ^3H - δ -aminolevulinic acid and myosin prelabeled with ^3H -leucine. C-control, H-end of 6 hours of hypoxia, H+G, 6 hours after return to normal O_2 tensions. From Aschenbrenner et al. (9).
- Fig. 5. Cardiac cytochrome c content after a 6-hour period of hypoxia, and 12 and 18 hours after return to a normal O_2 atmosphere. From Aschenbrenner et al. (9).
- Fig. 6. Incorporation of 1-hour pulses of ^3H -leucine into mitochondrial protein during the 6-hour period of 4-5% O_2 and after return to a normal atmosphere. From Aschenbrenner et al. (9).
- Fig. 7. Effect of banding of the ascending aorta of rats on total cardiac cytochrome c content, and on cytochrome c specific radioactivity, corrected for changes in cytochrome content. Cytochrome c was pre-labeled with ^3H - δ -aminolevulinic acid 3 days prior to aortic banding. From Albin and Rabinowitz (10).

Table I

Relative Incorporation of ^{14}C -amino Acids into Protein of Subcellular
Fractions of Hearts of Banded and Control Animals

Fraction	Sham	Banded	Ratio: $\frac{\text{Band}}{\text{Sham}} \times 100$
Homogenate	1.0	1.0	
Mitochondria	0.95	1.24	130.5
Myofibrils	0.97	0.90	93.0
Supernatant	1.28	1.35	105.5

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Fig 1

Fig 2

Effect of Hypoxia on The Turnover of Rat Heart Mitochondrial Proteins Labeled With ^{14}C Arginine

Fig 3

Change in specific activity after hypoxia treatment

Fig 4

Cytochrome c content of hearts after hypoxia

Fig 5

Incorporation of ^3H leucine into mitochondrial protein during and after exposure to hypoxia

Fig 6

Effect of aortic banding on cytochrome c
content and specific radioactivity

Fig 7.