6. Medes, G., Friedmann, B., Weinhouse, S., *Cancer Research*, 1956, v16, 57.
7. Noltmann, E., Bruns, F. H., *Biochem. Z.*, 1958, v330, 514.
8. Roe, J. H., *J. Biol. Chem.*, 1934, v107, 15.
9. Gomori, G., *Methods in Enzymol.*, v1, Academic Press, Inc., N. Y., 1955, p138.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.*, 1951, v193, 265.
11. Slein, M. W., *ibid.*, 1950, v186, 753.
12. Bruns, F. H., Noltmann, E., Willemsen, A., *Biochem. Z.*, 1958, v330, 411.

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Do Mitochondria Participate in General Cardiac Hypertrophy?* (25666)

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It is known that muscles which have a constant heavy amount of work, such as the breast muscles of birds have a very high density of mitochondria, while muscles with a lighter work load tend to have fewer mitochondria per unit of muscle mass(1,2). In muscles with a rich supply of mitochondria, it is possible that the increased number of mitochondria is determined by forces at work during embryological development, or that the increased number of mitochondria in these particular muscles is an adaptation to their heavy work load. It therefore seemed of interest to find out how much of an adaptive increase of mitochondrial mass occurs in a typical mammalian muscle which has undergone hypertrophy to meet an increased work load. Our model for muscle hypertrophy was the marked hypertrophy of the wall of the left ventricle which occurs in the rat following production of experimental renal hypertension.

Methods. A number of normal Wistar rats were divided into 2 groups. One group with 12 rats was left intact. This group had blood pressures ranging between 89 and 136 mm Hg. In the second group, one renal artery of each rat was narrowed with a silver clip. Six months later, 14 of the rats with the narrowed renal artery had developed moderate to severe hypertension, as determined by a microphonic manometer(3). Blood pressure was taken on at least 3 separate days within the week prior to killing the rat. The hypertensive rats with blood pressures ranging between 171 and 230 mm Hg were culled out to be compared with the completely normal, intact rats. Arterial pressures averaged 196 for hypertensive rats and 119 for normotensive rats. Our procedure for assessing degree of hypertrophy of the different fractions of the heart was as follows: The animal to be killed was anesthetized with ether and weighed and the heart promptly excised. All the blood was blotted from the cardiac cavities, and the right ventricular wall was trimmed away, as well as both atria, leaving only the left ventricular wall and the interventricular septum. The left ventricle which remained was quickly weighed on a torsion balance, and diced into cubes 1/16th of an inch in size. The cubes were then suspended in .25 molar sucrose containing .01 molar versenate in a Potter-Elvehjem homogenizer. The heart tissue was homogenized for 5 minutes while the homogenizing solution was kept at about 2°C. The homogenate and rinsings were then added to a precooled lusteroid tube, and spun in a Spinco Model L Centrifuge at 600 g for 12 minutes. The supernate from this spinning was transferred to another precooled lusteroid tube. The precipitate was resuspended in .25 molar sucrose and spun for another 15 minutes at 600 g. The supernate from this spinning was added to the other supernate fraction and the precipitate was again resuspended in .25 molar sucrose and the mixture spun at 600 g for one more time. This supernate was then added to the other 2 supernates to comprise the total mitochondrial fraction. The combined supernates containing the mitochondrial

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fraction were then spun in the Spinco Model L Centrifuge at 10,000 g for 20 minutes. The precipitate from this centrifugation was considered to be the genuine mitochondrial fraction. The supernate from this spinning was transferred to another precooled lusteroid tube, then spun for 15 minutes at 105,000 g. The precipitate from this spinning constitutes a sub-mitochondrial fraction which will be discussed below. The supernate from this centrifugation can be considered the "soluble" fraction. This fraction was then added to the original nuclear precipitate leaving 3 separate fractions: 1) the combination of nuclear and soluble fractions; 2) the genuine mitochondrial fraction; and 3) the sub-mitochondrial fraction. Trichloroacetic acid was added to all 3 fractions to provide a final concentration of 10% TCA. The trichloroacetic acid mixtures were shaken for an hour. After all protein had been precipitated, they were spun for 20 minutes at 2,000 rpm in an International Centrifuge. The supernates from all 3 of these fractions were decanted and discarded, and precipitates were heated in an oven at 110°C over night after the method of Hoch and Vallee(4). After heating, the precipitates were allowed to cool in a vacuum desiccator, then weighed on an analytical balance. The weights obtained provide an estimate of the protein content of the various fractions, as outlined in the method of Hoch and Vallee(4). In this system of analysis wet weight of the heart gives an estimate of total heart mass. The fraction which is a combination of the soluble and nuclear fractions gives an estimate of total amount of protein in the heart which is not present in any of the particulate fractions. This fraction will be designated as the "non-particulate fraction." The heated precipitate of the "genuine" mitochondrial fraction gives an estimate of amount of protein in the intact mitochondria. Amount of protein in the sub-mitochondrial fraction was also determined. The particles which make up this fraction are smaller than typical mitochondria. In the usual liver homogenate such particles would be called the "microsomal" fraction. However, heart muscle is said to contain fewer microsomes than liver(5). Ziegler *et al.*(6) have reported studies which suggest that certain sub-mitochondrial particles are primarily composed of fragments of mitochondria. Their evidence reasonably supports this contention. The sub-mitochondrial particles in our fractionation probably represent a mixture of microsomes, mitochondrial fragments and possibly other types of fragments and small particles. Total number of mitochondria in the mitochondrial suspension was determined by counting 4 samples in a Petroff-Hausser counting chamber. Total protein in the mitochondrial fraction was then divided by total number of mitochondria to get average mass of protein per mitochondrion.

Results. As would be expected, average heart weight:body weight ratio among the hypertensive group of rats was 55% greater than that of the normotensive group (Table I). Also, as would be expected, the "non-particulate" proteins of the heart were also increased per unit of body weight in hypertensive group compared to normotensive group. This ratio averaged 53% greater in hypertensives than in normotensives. The mitochondria also participated in the total hypertrophy of the heart. Total mitochondrial protein: body weight ratio was 25% greater in hypertensive rats than in normotensive rats. It is noteworthy that the mitochondrial fraction increased only 25% while the heart as a whole and the "non-particulate" proteins increased about 54% during the hypertrophy, resulting in a significant decrease in amount of mitochondrial protein per unit of left ventricular weight and per unit of "non-particulate" protein. The number of typical mitochondria in the mitochondrial suspension per 100 g of body weight was 50% greater in the hypertensive rats than in the normotensives ($p = .08$). The protein in the sub-mitochondrial particles also underwent a hypertrophy per unit of body weight in the hypertensive rats. This ratio was 37% greater in hypertensive rats than in normotensives. The hypertrophy was not as great, however, as that of the left ventricle as a whole or of the "non-particulate" protein.

The average mass of protein in an individual mitochondrion was somewhat smaller in hypertensive rats than in normotensive ones.

TABLE I. Relationships of Body Weight, Left Ventricular Weight and Mitochondrial and Sub-mitochondrial Proteins in Normal and Hypertensive Rats.

	12 normal rats (N)	14 hypertensive rats (H)	Ratio (H)/(N)	"p" value for difference between (N) and (H)
Left ventricular wt / Body wt	168	262	1.55/1.00	$< 1 \times 10^{-14}$
"Non-particulate" protein wt / Body wt	295	451	1.53/1.00	"
Mitochondrial protein wt / Body wt	498	622	1.25/1.00	"
Total No. of suspended mitochondria / 100 g body wt	5.67×10^{9}	8.50×10^{9}	1.50/1.00	.08
Sub-mitochondrial protein wt / Body wt	213	293	1.37/1.00	<.00001
Mitochondrial protein wt / Left ventricular wt	298	242	.81/1.00	<.00005
Mitochondrial protein wt / "Non-particulate" protein wt	169	140	.83/1.00	<.003
Sub-mitochondrial protein wt / Left ventricular wt	129	114	.88/1.00	.05
Sub-mitochondrial protein wt / "Non-particulate" protein wt	73	66	.90/1.00	.13
Avg wt of protein in single mitochondrion (μg)	11.2×10^{-6}	9.0×10^{-6}	.77/1.00	.16

However, the difference was not statistically significant.

Conclusions and summary. 1. Left ventricular weight:body weight ratio of a group of hypertensive rats was 55% greater than that of similar group of normotensive rats. 2. Ratio of "non-particulate" proteins of left ventricle to total body weight was 53% greater in hypertensive group than in normotensive group. 3. Mitochondria of left ventricle also participated in cardiac hypertrophy of the hypertensive group. Mitochondrial protein:body weight ratio was 25% greater in hypertensive group than in normotensive group. 4. Sub-mitochondrial particles of left ventricle, smaller in size than the mitochondria, also participated in cardiac hypertrophy. Ratio of protein in these particles to total body weight was 37% greater in hypertensive series than in normotensive series. 5. Hypertrophy of mitochondria, however, was not as great proportionately as that of left ventricle as a whole. Ratio of mitochondrial protein to total left ventricular weight was 19% less in hypertensive rats than in normotensive group. When expressed as ratio of mitochondrial protein to "non-particulate" protein, the ratio was 17% less in the hypertensive group compared to normotensive group. 6. Hypertrophy of the sub-mitochondrial particles in hypertensive rats was also proportionately less than the hypertrophy of left ventricle as a whole. 7. The mass of protein in individual mitochondrion was slightly less in hypertensive rats than in normotensive group, but the difference was not significant. Total number of mitochondria increased 50% as the left ventricle hypertrophied ($p = .08$). 8. It seems clear that the total mass of mitochondrial protein in the left ventricle can increase in adaptation to an increase in work load.

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1. Lindberg, O., Ernster, L., *Chemistry and Physiology of Mitochondria and Microsomes,* Springer-Verlag, Vienna, Vol. 3, 1954.

2. Paul, M. H., Sperling, E., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 352.

3. Friedman, M., Freed, S. C., *ibid.,* 1949, v70, 670.

4. Hoch, F. L., Vallee, B. L., *Analyt. Chem.*, 1953, v25, 317.

5. Slater, E. C., *Sarcosomes (Muscle Mitochondria), Symposia of Soc. for Exp. Biol.*, 1953, v10, p113.

6. Ziegler, D. M., Linnane, A. W., Green, D. E., Dass, C. U. S., Ris, H., *Biochim. Biophys. Acta*, 1958, v28, 524.

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Development of Lipemia in Rats Following Intravenous Injection of India Ink.* (25667)

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In the course of studies of the role of the liver in destruction of clearing factor, Higgins' India Ink was injected into rats. It was observed that blood samples withdrawn one to 2 hours after injection were lactescent. This lactescence was associated with an increase in level of total fatty acids. The lipemia occurred in fasted rats thus indicating that the India Ink was either stimulating an increase in rate of mobilization of fat from the depots or blocking the exit of the mobilized fat from the blood stream. Since the reticuloendothelial system is readily saturated with the India Ink particles, the lipemia could have resulted from inhibition of the normal function of these cells. It has been reported (1) that the reticuloendothelial system functions in uptake of cholesterol from the blood. The evidence(2,3) is against these cells playing any major role in uptake of chylomicrons. The following experiments were carried out in an attempt to determine the mechanism by which India Ink induced lipemia.

Methods and materials. Total fatty acids. The lipids were saponified by heating in presence of alkali and alcohol. The alcohol was removed and the fatty acids were freed by addition of acid in excess. The fatty acids were transferred to a solvent and, after evaporation of the solvent, were titrated by standard alkali. The details were as follows: 0.2 ml of plasma was added to 5 ml of alcohol-ethyl ether mixture (3:1 v/v) and heated to boiling in a water bath. The mixture was then cooled and filtered into a graduated test tube. The residue was reextracted, filtered into the same test tube, and total volume of the extracts made up to 12.0 ml. Five ml of the filtrate was placed in a 15 x 85 mm test tube with screwcap stopper and 0.1 ml of saturated NaOH was added. The mixture was heated in a boiling water bath for 30 min. It was then cooled and while cooling sufficient 33% sulphuric acid solution was added to give an acid reaction. Six ml of petroleum ether was added. The tube was stoppered tightly and shaken vigorously for 1 minute; then 3.5 ml of water was added and the tube shaken again. The mixture was allowed to settle, then two 2 ml aliquots of the clear supernatant were placed in 15 x 85 ml test tubes. The solvent was removed by evaporation and 3 ml of a 0.004% alcohol solution of thymol blue was added. Titration was carried out with 0.02 N KOH. Results are expressed in reference to a standard solution of oleic acid (100 meq/liter).

India Ink. Higgins' India Ink was obtained from the Higgins' Ink Co. Chin Chin India Ink and Pelikan Carbon (C11/1431a) were obtained from the Heinz Jordan and Co. Ltd. These were diluted 3:5 with saline and 1 ml was injected into the exposed jugular vein of each rat. Unless otherwise stated the term India Ink will refer to Higgins' India Ink only.

Rats. The animals used were female rats of the Wistar strain weighing 200-250 g, maintained on a standard chow diet and fasted over-night prior to injection.

Protamine and heparin were supplied by the Connaught Medical Laboratories, Toronto.

Results. In the first experiment India Ink

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