PROTEIN : LIPID RATIOS OF LIVER MITOCHONDRIA DURING DEVELOPMENT

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In the course of a series of experiments utilizing mitochondria from embryonic chick livers, it was found that the density of these mitochondria increased during development. The change was of sufficient magnitude that the two extremes of age could be resolved in a continuous sucrose density gradient (Fig. 1). This suggested that the protein: lipid ratio might increase with age. Estimation of total protein and total lipid of mitochondrial pellets from early embryos, posthatching chicks, and adult chickens has borne this out (Table I). During this time the protein:lipid ratio increases by a factor of approximately two and is highest in the posthatching chick.

The majority of reports on the composition of whole liver mitochondria have given values between 21 and 29.6% for the proportion of total lipid (see review by Ball and Joel, 1962). If it is assumed that the lipid plus protein account for at least 90% of the liver mitochondrial dry weight (Ada, 1949), the minimum protein:lipid ratio can be calculated to vary between 2.0 and 3.3. The mean value reported here for adult chickens fits within this range. However, the value of 4.7 for posthatching chicks is far higher than any previously reported. The reason for this is unknown, but two possibilities should be considered. First, because almost all of the mitochondrial lipids are membrane bound so that the contribution of the matrix is largely protein (Lehninger, 1964), it is conceivable that at the posthatching stage of development liver mitochondria may have a large amount of matrix but may be relatively poor in cristae as compared with liver mitochondria at the adult stage. Second, the mitochondrial membranes themselves may be relatively rich in protein at this stage and may contribute at least partially to the

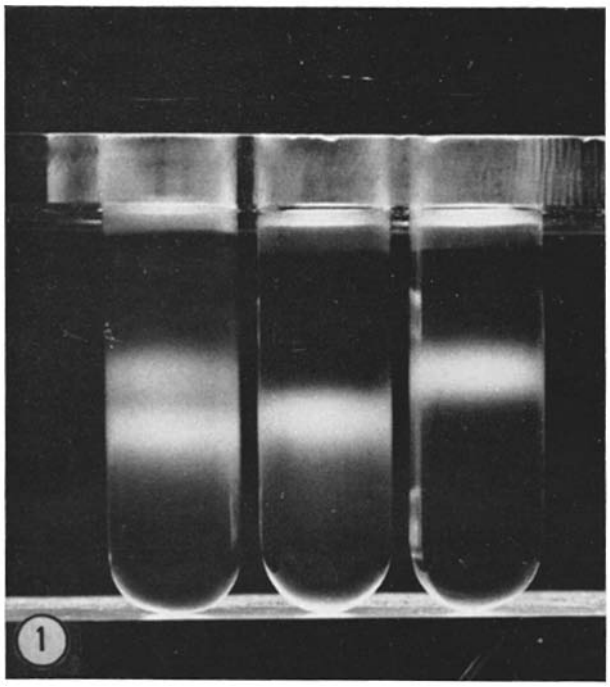

FIGURE 1 Density differences between embryonic and adult chicken liver mitochondria. The left-hand tube contains an artificial mixture of mitochondria from an adult and from embryos of 8 days of incubation. The center tube contains mitochondria from an adult. The right-hand tube contains mitochondria from embryos of 8 days of incubation. Livers were weighed and gently homogenized in nine parts 0.44 M sucrose (0.001 M EDTA) in a glass homogenizer with a Teflon pestle. The heavy cellular components of this homogenate were centrifuged down at 750 *g* for 15 min. The supernatant fluid was gently removed, weighed, and centrifuged at 5,100 *g* for 15 min. The resultant mitochondrial pellets were resuspended in a small amount of 0.88 M sucrose (0.001 M EDTA). 300 μl of such a suspension were layered over a 4 ml continuous gradient of 1.02–1.75 M sucrose (0.001 M EDTA). The gradients were centrifuged for 2 hr at 15,000 rpm (18,400 *g* at R_{av}) in the SW39 rotor of a Spinco Model L preparative ultracentrifuge. 12 hr of further centrifugation at this speed did not change the position of the bands. All stages of the experiment, after dissection of the livers, were carried out at 0°C, and all solutions were at pH 7.4.

TABLE I

Protein: Lipid Ratios of Whole Liver Mitochondria Isolated from Chick Embryos and Adults

Age	Protein:lipid ratios	Mean
8 days of incubation	1.75, 1.8, 1.9, 2.2	1.9
9–11 days of incubation	2.3, 2.4, 2.5, 3.0, 3.0	2.65
1–14 days posthatching	4.25, 4.75, 4.96, 4.96	4.73
Adult	2.7, 3.1, 3.8	3.2

Each value represents one set of determinations on a single mitochondrial preparation. Adult preparations were derived from a single animal; all others represent pooled livers from a number of animals. Mitochondrial pellets were resuspended by homogenization in a small amount of distilled water. Aliquots of this suspension were taken for the two determinations. Protein was measured by the method of Lowry et al. (1951) with bovine serum albumin as a standard. The Lowry method was checked by the method of Warburg and Christian (1942) on duplicate samples and found to be accurate for the material in question. Lipids were extracted according to the method of Folch et al. (1951, 1957), by use of 0.04% chloride to wash the initial chloroform-methanol extract (Luck, 1965).

high protein: lipid ratio of the whole mitochondrion.

Similarly, the very low protein:lipid ratios of the early embryo may indicate mitochondrial membranes of unusually high lipid content, but they may also show the very small amount of matrix material present in such mitochondria. Stephens (1965) has prepared a series of excellent electron micrographs of chick embryo liver. These show very clearly the extreme hydration of early embryonic mitochondrial matrix material, which appears to have large "holes" or areas of almost complete electron lucidity. With age, the matrix material can be seen to become more uniform and dense.

In their review, Ball and Joel (1962) present from a number of laboratories, data on protein: lipid determinations of mitochondrial membrane preparations of different sorts, from both heart and liver. The protein:lipid ratios of these membranes appear to range from 2.3 to 2.7. It thus seems likely that, since the protein:lipid ratio of whole liver mitochondria from 8-day embryos is lower than that of liver mitochondrial membrane from adults, the early liver mitochondrial membrane has a comparatively high lipid content.

Whether these changes represent the differentiative pathway which must be followed in order to produce the specialized adult liver mitochondrion or, rather, the possibility of differential mitochondrial function at the various stages of development (Henson, 1967) is a question of importance for those investigators interested in the mechanisms of mitochondrial control. However, the less interesting possibility that these ratios signify only the apparent ability of mitochondria to incorporate widely varying types and amounts of lipids, depending on the latter's presence in the nutritive material (Luck, 1965), should also be considered.

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