THE STRUCTURE OF RAT LIVER MITOCHONDRIA: A REEVALUATION

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SUMMARY

Mitochondria have been considered to have single spherical or tubular shape. Recently a new model has been proposed for the structure of yeast mitochondria which suggests that there is a single, branched, tubular mitochondrion per cell. Using the technique of serial sectioning, we present evidence showing that some, but not all mitochondria of normal adult rat liver have a branched, tubular structure. These observations should lead to a re-evaluation of current concepts regarding mitochondrial function and biogenesis.

INTRODUCTION

The traditional concept of a liver mitochondrion is that of a small, discrete, intracellular organelle relatively free in the cytoplasm which is in some manner capable of reproducing itself. The typical mitochondrion of the hepatic parenchymal cell of the adult rat is usually described as a rod approximately 0.3 μ in diameter and 1 μ in length (1). Stempak (2) using serial sections, described an unusual form in neonatal rat liver. The mitochondria were described as essentially biconcave disks or planoconcave disks with nipple-like projections from the outside surface of the disk. More recently Berger (3) proposed a model in which there were two populations of mitochondria, one corresponding to the classical rod shaped configuration and the other a larger V-shaped configuration. Although rat hepatocytes have been reported to have from 500 to 2500 mitochondria, an average of 800 per cell has been suggested (1).

Recently Hoffmann and Avers (4), working with yeast, presented evidence for a single mitochondrion per cell. Using serial sectioning techniques they showed that all the mitochondrial profiles were cross sections through a single

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branching, tubular structure which upon cell division was split between the two daughter cells. Since this concept radically alters ideas concerning mitochondrial function and biogenesis, we have reevaluated mammalian mitochondrial structure using serial sectioning techniques to determine if they have a structure similar to that proposed for yeast. Preliminary evidence indicates that there is a high degree of branching present in some, but not all of the mitochondria of normal adult rat liver.

MATERIAL AND METHODS

Male rats (Charles River) weighing approximately 225 to 250g were used for all experiments. The animals were fasted overnight and sacrificed by carotid exsanguination. The liver was quickly removed and small sections fixed in 2% glutaraldehyde buffered to pH 7.4 (6-8 hours) and postfixed in osmium tetroxide (5). Following dehydration in graded concentrations of ethanol the tissue was embedded in Epon 812 and ultrathin sections, approximately 0.08 micron were prepared using a Porter-Blum Ultramicrotome. Series of up to thirty-five successive sections were used for the analysis. The sections were stained with lead hydroxide and uranyl acetate. Serial sections were examined and photographed with a Hitachi HS-7 electron microscope. Scale models were reconstructed from cardboard with the use of clear plastic overlays.

RESULTS AND DISCUSSION

Evidence of branching was seen in every cell examined, but branching was not observed in every mitochondrial profile. Types of configurations observed included simple rod shapes, longer filamentous rods, V-shaped mitochondria, disk-shaped mitochondria, and more complex branched, tubular structures. The rod and V-shaped mitochondrial profiles had dimensions which correspond to those described by Berger. The disk-shaped mitochondria were usually mono-concave disks with a diameter ranging from 1.2 to 1.5 μ. The total dimensions of the more complex forms were not determined; however, the diameter of any tubular component corresponded to the diameter of the other forms seen. Thus, in any given section, several rod to ovoid mitochondrial profiles may be part

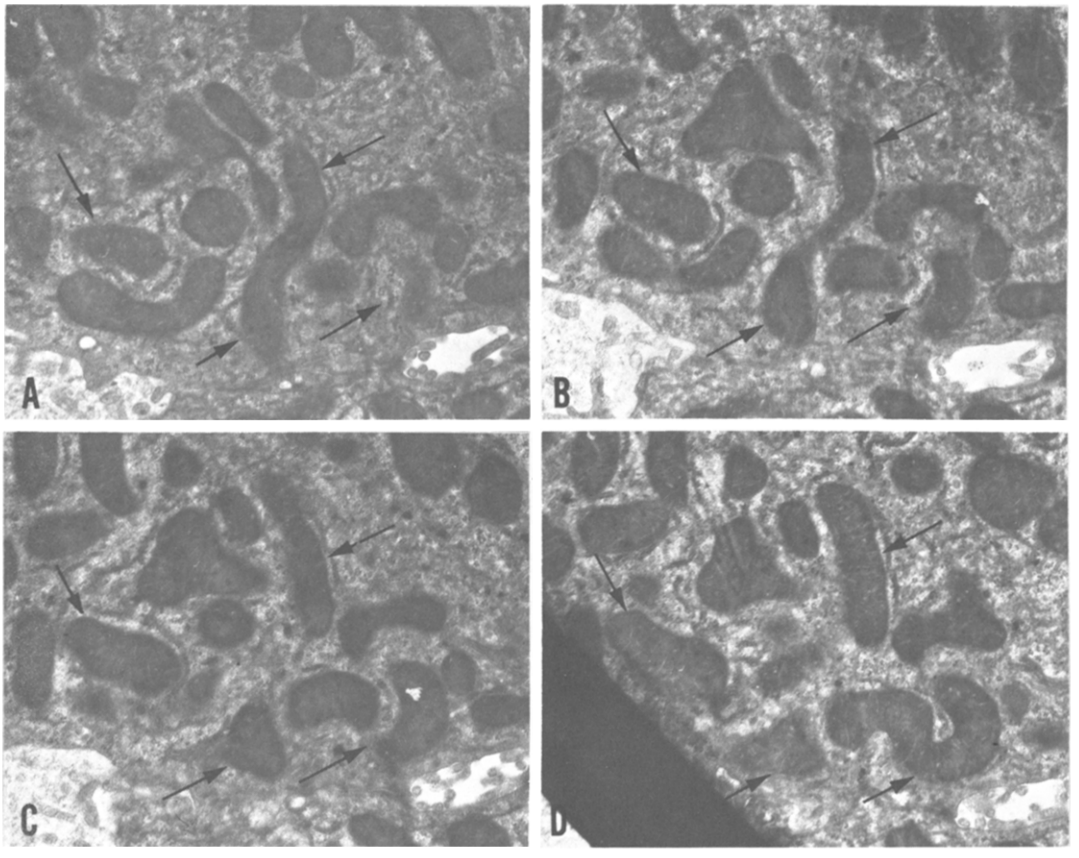

Figure 1: The first four successive (A-B) sections through a cell showing components of a single mitochondrion as demarcated by the arrows.

of the same mitochondrion, as seen in Figure 1 (C).

Figure 1 shows eight successive sections through a cell. A branched, tubular mitochondrion is demarcated by the arrows. In Figure 1 C, five independent components are seen. The relationships of these components to each other and the surrounding mitochondria are quite evident. Two views of a scale model of this mitochondrion are seen in Figure 2.

A model of another complex mitochondrion is shown in Figure 3. It consists of numerous tubular structures which are joined together. Other mitochondria observed appeared to be closely related and in the process of fusion or fission. The variety of forms seen and the types of interrelationships observed suggest

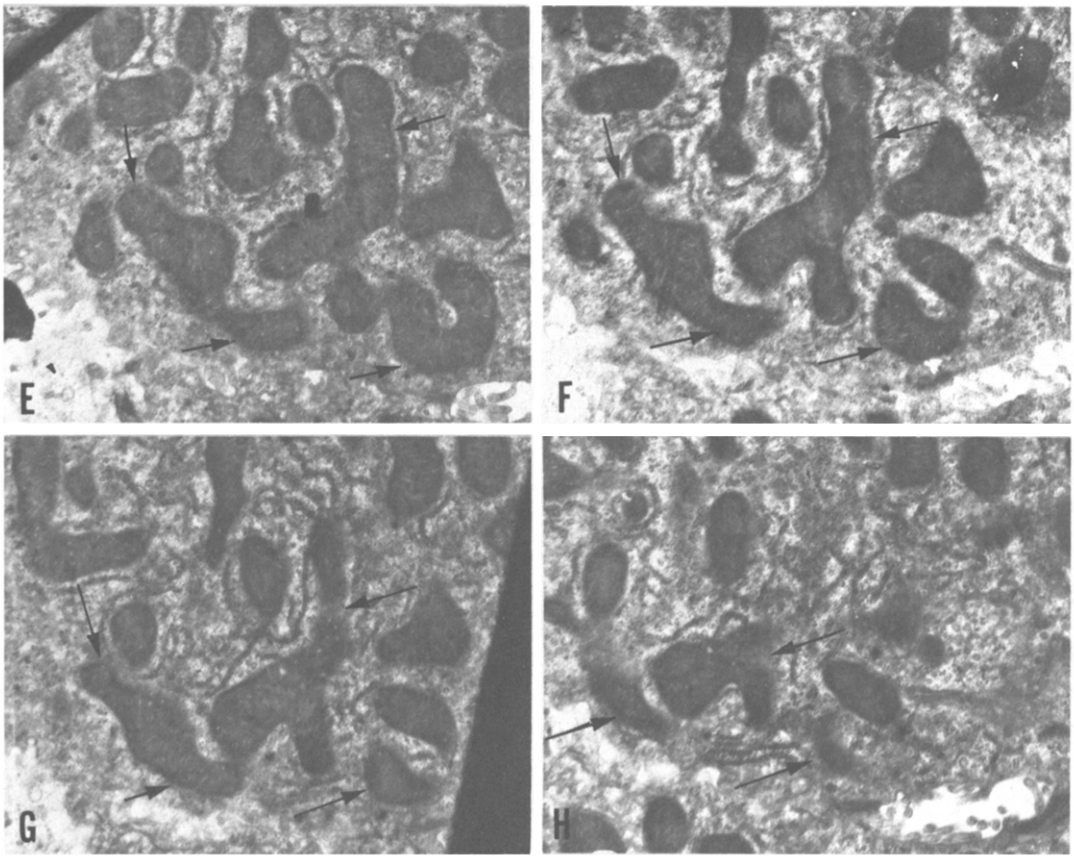

Figure 1 (Continued): Second four successive (E-H) sections showing the continuation of the mitochondrion as demarcated by the arrows. Total magnification before reproduction: 9,600.

that the structure of hepatocyte mitochondria may be quite plastic. How such a model would affect function, particularly of the membranes, is a problem which needs to be readdressed.

Hoffmann and Avers (4) found that the basic structure of the mitochondrion in yeast remained the same during various nutritional states, with only the diameter of the tubule changing. Preliminary results in this laboratory suggest that rat liver mitochondria, under varying experimental conditions undergo marked structural changes which are more complex than reported for yeast. The variability in the structure of mitochondria within and between various cells types, and under changing nutritional states, raises basic

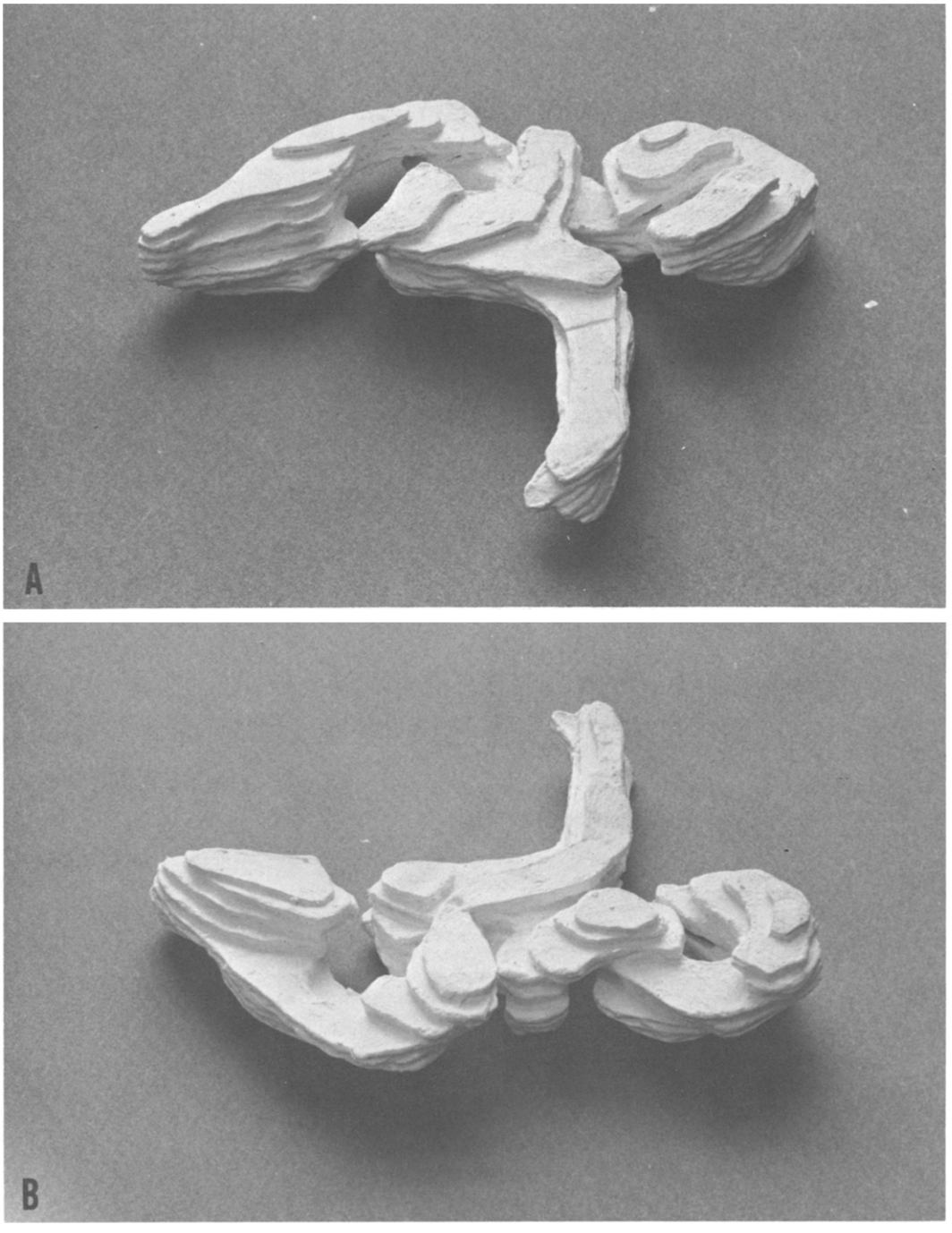

Figure 2: Scale model of the mitochondrion shown in Figure 1. The 3-dimensional relationships are apparent.

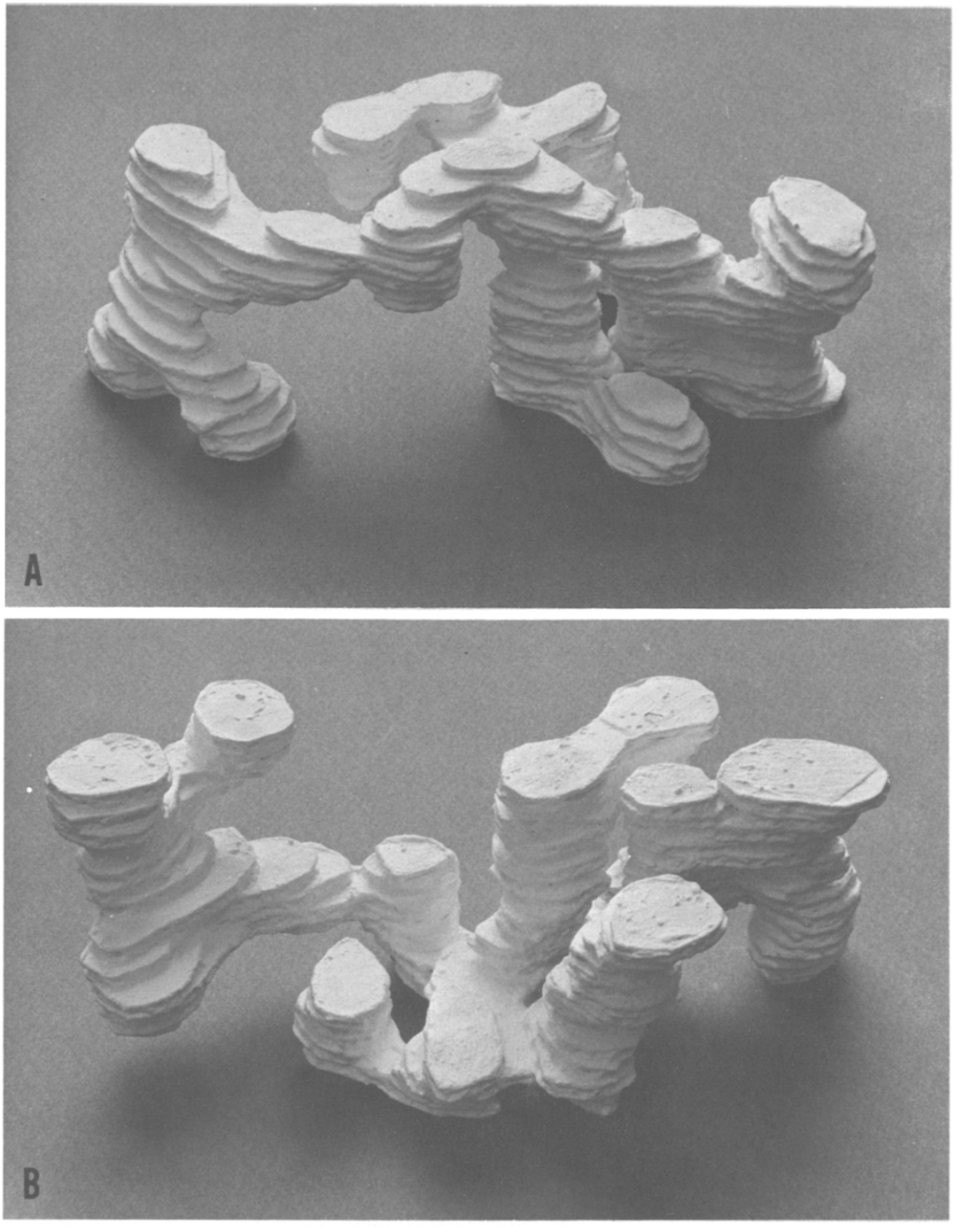

Figure 3: Scale model of a complex mitochondrion. It appears to be composed of several tubular segments which have joined together.

questions concerning not only the organization and control of intracellular structure but also offers a model for the study of these mechanisms, and how they may relate to various physiologic and pathologic states.

Our results indicate that there are a variety of configurations of mitochondria present within normal rat hepatocytes. The concept of one mitochondrion per cell as in yeast (4) does not appear to be the case in rat hepatocytes, but the model of highly branched mitochondria does apply. These observations indicate that a reevaluation of current concepts regarding mitochondrial function and biogenesis is needed. Studies on mitochondrial biogenesis in mammalian systems which were based on the premise of a uniform population of mitochondria relatively free in the cytoplasm should be reassessed.

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