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Ultrastructural Changes in Fibroblast Mitochondria of a Patient with HHH-Syndrome[1]

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Electron micrographs of fibroblasts of an HHH-syndrome patient showed abnormal structures, similar, but not identical, to those observed in the liver of such patients. It is suggested that incorporation of a mutated protein into the inner mitochondrial membrane gives rise to a rearrangement of that membrane, resulting in unusual structures.

The HHH-syndrome [hyperornithinaemia, hyperammonaemia, homocitrullinuria syndrome (McKusick 23897)], a rare autosomal recessive disease of the urea cycle, is possibly caused by a defective transport of ornithine across the inner mitochondrial membrane (Fell *et al.*, 1974; Shih *et al.*, 1980, 1982; Hommes *et al.*, 1982; Gray *et al.*, 1982). Gatfield *et al.* (1975) and Haust *et al.* (1981) identified, by ultrastructural studies of hepatic tissue, obtained at biopsy from a patient with the HHH-syndrome, mitochondria of abnormal shape and unusual features. Tubules extending throughout the length of large mitochondria were observed, arranged in a rosette-like manner. It has been speculated that these unusual membrane structures contribute to the decreased ability of ornithine to reach the matrix space of the mitochondria (Haust *et al.*, 1981). Several studies have indicated that the basic defect of the HHH-syndrome is expressed in fibroblasts (Shih *et al.*, 1980, 1982; Gray *et al.*, 1982; Hommes *et al.*, 1982). The question arises therefore whether such abnormal structures can be demonstrated in fibroblast mitochondria.

MATERIALS AND METHODS

Skin biopsies were obtained with informed consent. Fibroblasts were grown in minimal Eagle's medium, supplemented with 10% (v/v) fetal calf serum, 100 U ml^{-1} penicillin and 100 µg ml^{-1} streptomycin. The cells were harvested at confluence by trypsinization. The HHH-syndrome patient whose cells were used in the present study has been described elsewhere (Hommes *et al.*, 1984). Control cells were obtained from seven healthy males.

For electron microscopy, the pelleted cells were fixed in 4% glutaraldehyde in phosphate buffer, postfixed in 1% osmium tetroxide for 1 h. The cells were dehydrated in ascending concentrations of ethanol and embedded in Epon. Ultrathin sections were made with an MT-5000 ultramicrotome and stained with freshly prepared lead citrate and uranylacetate and examined in a Philips transmission electron microscope. Stereological measurements to determine the volume fraction of nucleus and mitochondria were carried out by application of the Delesse principle (Delesse, 1847; Weibel and Elias, 1967). To this end the area of a cell was redrawn on transparent paper, cut out and weighed. Then the areas of the nucleus and mitochondria were indicated on the paper, cut out and weighed. The percentage of surface area occupied by the nucleus and mitochondria were then calculated. These percentages are equal to the volume percentage for these cellular compartments, provided a statistically sufficient number of sections is examined (Weibel and Elias, 1967).

RESULTS

Electron micrographs of control and patient fibroblasts are shown in Figure 1. Both cells contain numerous autophagic vacuoles, which are known to occur in fibroblasts harvested at confluency (Comings and Okada, 1970; Lucky *et al.*, 1975). The mitochondria were mostly in the condensed configuration, both in the control cells and in the patient's fibroblasts.

Stereological measurements for the volume fraction of the nucleus showed no statistically significant differences between control cells and patient cells (Table 1). Applying Student's *t*-test, a P value of $0.20 < P < 0.25$ can be calculated. Control cells showed in general a smaller volume percentage for the mitochondria than the cells of the HHH-syndrome patient.

No evidence could be seen for the occurrence of bizarrely shaped mitochondria, such as observed in the liver of HHH-syndrome patients. Particularly, no tubular structures extending thoughout the length of large mitochondria were observed, which have been described as characteristic for liver mitochondria of HHH-syndrome patients (Haust *et al.*, 1981). However, in some areas of the patient's mitochondria triangular

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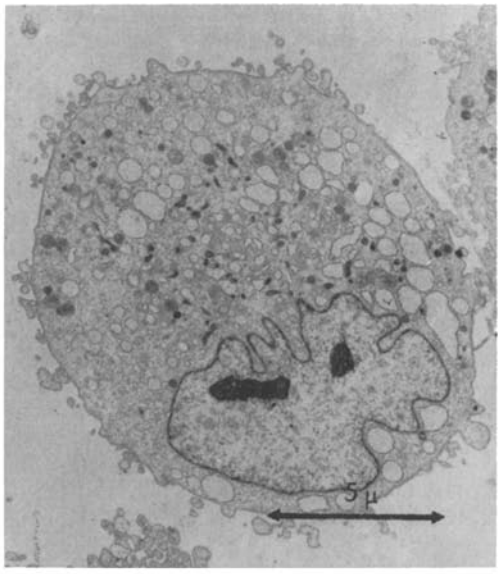
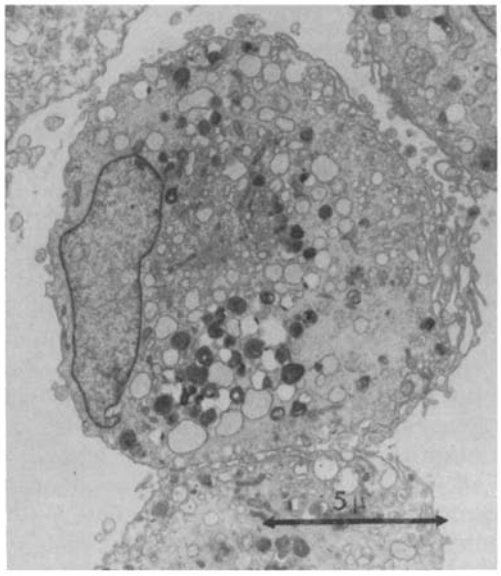


(A)



(B)

Figure 1 Morphology of control fibroblasts (A) and HHH-syndrome patient fibroblast (B) at low magnification

structures were observed (Figure 2B), though not observed in mitochondria of the control cells (Figure 2A), which resemble the cross-sections of the triangular tubules observed in liver mitochondria.

Loosely laminated figures, resembling myelin figures, are normally observed in electron micrographs of fibroblasts (Comings and Okada, 1970; Lucky *et al.*, 1975). The number of such bodies per cell is, however, considerably higher in the patient's fibroblasts than in fibroblasts of control 1: 25.6 ± 14.9 vs. 12.1 ± 7.2 (mean value ± SD), which is a statistically significant difference ($P < 0.0025$, applying Student's *t*-test). Similar differences were observed when the other control cell lines were compared with the patient's fibroblasts.

Table 1 Stereological measurements for volume fractions of the nucleus and of mitochondria of control fibroblasts and of fibroblasts of an HHH-syndrome patient

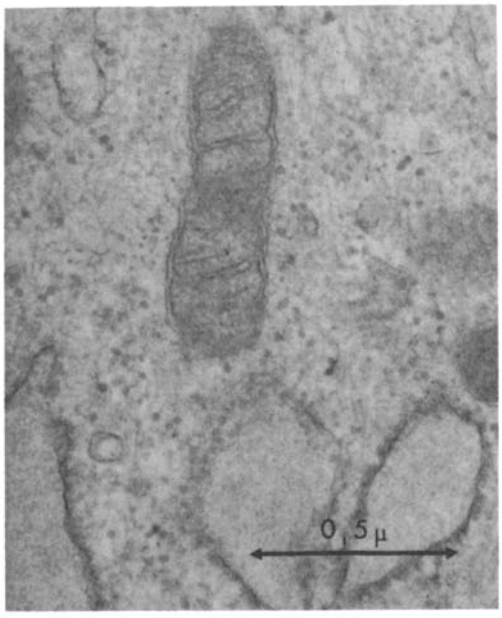
Cell line	*Nucleus*	*Mitochondria*
Control 1 (16)	17.85 ± 5.95	2.83 ± 1.53*
Control 2 (14)	12.72 ± 10.29	1.67 ± 0.59*
Control 3 (7)	22.08 ± 10.99	1.53 ± 0.90†
Control 4 (11)	19.68 ± 11.73	1.05 ± 0.76†
Control 5 (13)	21.80 ± 11.11	1.31 ± 1.05†
Control 6 (12)	22.47 ± 11.95	2.12 ± 0.90*
Control 7 (9)	20.50 ± 6.28	1.01 ± 0.40†
HHH-patient (16)	15.90 ± 7.96	3.67 ± 1.97

Values are expressed as percentages of the total cellular volume ± SD, with the number of electron micrographs examined per cell line in parenthesis
* $0.05 < P < 0.10$ when compared with mitochondrial volume fraction of HHH-patient applying Student's *t*-test
† $0.005 < P < 0.01$ when compared with mitochondrial volume fraction of HHH-patient applying Student's *t*-test

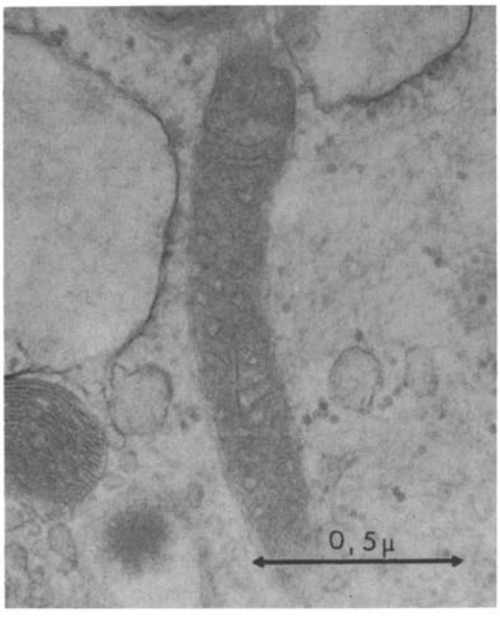
DISCUSSION

Several studies have provided evidence that the basic defect of the HHH-syndrome is expressed in fibroblasts (Shih *et al.*, 1980, 1982; Gray *et al.*, 1982; Hommes *et al.*, 1982). Nevertheless, such dramatic changes in the morphology of mitochondria, as observed in liver mitochondria, could not be seen in fibroblast mitochondria, except for triangular structures, resembling those seen in liver mitochondria. Whether the origin of these abnormal structures is the same in both types of cells remains to be established.

It is interesting to note that in several diseases where a deficiency of a protein constituent of the inner mitochondrial membrane has been described, mitochondria with an abnormal shape and structure have been found. Examples include cytochrome aa_3 deficiency (Van Biervliet *et al.*, 1977; DiMauro *et al.*, 1980; Prick *et al.*, 1983), cytochrome *b* deficiency (Morgan-Hughes *et al.*, 1977), Luft's disease (Luft *et al.*, 1962; Haydor *et al.*, 1971), defects in the respiratory chain-linked energy transfer reactions (Schotland *et al.*, 1976), NADH-CoQ reductase deficiency (Land *et al.*, 1981), lack of respiratory control of skeletal muscle mitochondrial α-glycerophosphate oxidation (DiMauro *et al.*, 1973), as well as the HHH-syndrome (Haust *et al.*, 1981). In all of these cases the defects are only partial. Presumably, a mutated protein is therefore present in the inner mitochondrial membrane. Such mutated proteins with a different tertiary structure may not fit properly in the inner mitochondrial membrane, resulting in a rearrangement of this membrane, which manifests itself as unusual structures. There is, admittedly, no proof for this hypothesis, except that the unusual structures are a common feature of inner mitochondrial membrane defects. It has been shown that uncoupling with dinitrophenol can result in abnormal structures of skeletal muscle mitochondria (Sahgal *et al.*, 1979). Elimination of the proton gradient with concomitant loss of respiratory control can therefore equally lead to abnormal mitochondria. In this



(A)



(B)

Figure 2 Longitudinal section of mitochondria of fibroblasts of control (A) and HHH-syndrome patient (B)

context, it should be noted that the matrix pH of HHH-patient fibroblast mitochondria is not different from that of controls (Metoki and Hommes, 1984).

The loosely laminated bodies are also generated by the dinitrophenol treatment. These figures are derived from mitochondria in view of the high NADH-oxidase activity (Sahgal *et al.*, 1979). The fibroblasts of the HHH-syndrome patient contain increased amounts of these bodies. That would be consistent with abnormal structures of the mitochondria.

In a recent review, Sengers *et al.* (1984) have summarized the mitochondrial myopathies. It was concluded that in all likelihood the abnormalities of the mitochondria are secondary phenomena, a conclusion in agreement with the proposal made here.

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Book Review

Metabolic and Endocrine Emergencies – Recognition and Management, 2nd edn.
Edited by Habeeb Bacchus. University Park Press, Baltimore, $25.00.

This is the second edition of a book, published first in 1976, which aims to teach the clinician how to recognise and treat metabolic and endocrine emergencies. The book is soft covered and just about pocket sized. There are 252 pages with 19 pages of adequate index. The print and diagrams are clear and the chapter subdivisions boldly headed for easy reference.

There are 23 chapters, of which the first is the most useful. It discusses metabolic and endocrine emergencies in terms of clinical presentation and urgent laboratory investigations rather than diseases. In contrast, remaining chapters cover the recognition, differential diagnoses, management and pathogenesis of specific diseases. There are chapters on metabolic acid–base disturbances, disorders of glucose metabolism, disordered sodium, potassium and calcium states and porphyria. Much attention is paid to the effect of renal insufficiency on the disorder of glucose metabolism. There are instructive chapters on lactic acidosis and alcoholic ketosis, and also a very good new chapter on hypophosphataemia, a topic poorly dealt with in other books. The endocrine emergencies are represented by acute adrenal insufficiency, congenital adrenal hyperplasia, hyperthyroid storm, myxoedema coma and phaeochromocytoma crisis.

The main strength of this book is its concentration on developing a thorough understanding of the basic pathophysiology and rationale behind management. Much of the basic information can be found in standard textbooks but it is the attention to detail and explanation that makes this book stand out. There is, however, little new and controversial to be found which makes the already concise text dry and unimaginative at times. In content, this book falls somewhere between the standard texts and up-to-date reviews, such as the *Clinics of North America* series.

Although not intending to be, the book is to some extent a didactic cookbook of emergency management. For some users of the book that will be an advantage, but it does mean that no allowance is made for management differences across the North Atlantic, and these could cause confusion to the unwary. For example, why should cows' milk be avoided in neonatal hypocalcaemia? and it is disappointing that there is no discussion or even mention of 1-α-hydroxy-cholecalciferol. The text also, at times, fails to maintain its usual clear and detailed explanations. The concept and clinical relevance of pseudohyponatraemia caused by hyperglycaemia is difficult to understand and the author remains undecided when advising how much bicarbonate to use in treating acidosis; at one point he advises 60–100% of total body deficit and later in the same paragraph 4–6 mEq/litre.

Except for diabetes and its variants, the conditions covered by this book are fairly rare. The book contains a wealth of information, including detailed management regimes. Much of this is available elsewhere, but not concentrated in a single, handy volume. This, I feel, will be the book's main appeal: to the clinician in training for understanding the subject or actual reference while managing a patient and to the general clinician for brushing up his knowledge. It can be recommended as a book for reference but not necessarily to own.

M. Bain