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Isolated mitochondria from frozen muscle have limited value in diagnostics

Received: 31 May 1994
Accepted: 20 June 1994

Sir: Applegarth, Tong and Clarke [1] reported that the activities of citrate synthase, NADH-cytochrome c reductase, succinate-cytochrome c reductase, and succinate-CoQ reductase were not significantly different in mitochondria isolated from fresh and frozen muscle, while the activity of complex I was 16% higher and that of complex IV 16% lower in preparations from frozen muscle. The authors stated that investigation of mitochondria from frozen muscle is as useful as from intact muscle. This is not correct. When one is only interested in the activities measured by Applegarth et al. [1], there is no need to isolate mitochondria, because these activities can better be studied in a homogenate from (frozen) muscle.

Mitochondria from frozen muscle are different from freshly isolated mitochondria, in properties, yield and purity. They do not catalyse oxidative phosphorylation, because the protein gradient, the driving force for ATP synthesis, cannot be built up due to destruction of the inner mitochondrial membranes. Complex V catalyses ATPase instead of ATP synthetase. Mitochondrial enzymes from intermembrane and matrix space are solubilized. The fact that some mitochondrial activities were the same in the mitochondrial preparations from fresh and frozen muscle is just accidental. The activities were calculated per milligram protein, but the composition of the protein is different in the two preparations. Isolated mitochondria from frozen muscle are suitable for the determination of cytochrome redox spectra [2].

Trijbels et al. [3] advocated to study in patients suspected from having a defect in oxidative phosphorylation and/or pyruvate dehydrogenase complex, both oxidative phosphorylation in a preparation with intact mitochondria and all candidate enzymes in a homogenate from frozen muscle, to enable the detection of all possible defects present. Several groups investigate only isolated mitochondria from freshly biopsied muscle or homogenates from frozen muscle. In the view of Trijbels et al. [3], the best is to investigate both. Unfortunately this is not possible in all patients, and this will give rise to underdiagnosis of mitochondrial defects, and to a wrong diagnosis.

A nice example of using the combined diagnostic approach is the finding of a defect in the adenine nucleotide carrier [4], which was detected by an immunological method in frozen muscle. The suspicion of a deficiency of this translocator was raised when a phosphorylation defect was detected in oxidative phosphorylation by intact muscle mitochondria.

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Reply

Received, accepted: 20 June 1994

Sir: We thank Drs. Scholte and Trijbels for pointing out some important considerations concerning our report on the measurement of electron transport chain activities in frozen versus fresh muscle. We agree that when possible the use of fresh muscle is preferable over that of frozen. Fresh muscle allows for the evaluation of oxidative phosphorylation in intact mitochondria and can point to a defect in complex V and the adenine nucleotide carrier, etc. Unfortunately many patients are seen and evaluated at centres that cannot provide as thorough an evaluation of muscle as could be performed by Scholte and Trijbels.

It has been our experience that many patients found to have lactic acidosis with normal activities of pyruvate dehydrogenase complex and pyruvate carboxylase in fibroblasts most commonly have defects in complexes I, II, III or IV of the electron transport chain. We have found it most convenient to be able to identify these defects by using mitochondria isolated from muscle frozen for 7 days for the reasons we gave in our original letter to the Editor. We agree with Scholte and Trijbels comments on the effect of freezing on some mitochondrial enzymes but dispute their conclusion that the similar results for complexes I, II, III and IV for mitochondria isolated from otherwise identically treated samples of fresh or frozen muscle were just accidental.

When we have isolated mitochondria from muscle samples which have been frozen for more than roughly 3 months we get low activities of the complexes we described and low activities of citrate synthase which can act as a marker enzyme against which we can assess, crudely, the reliability of the other complex activities. However, once mitochondria have been isolated under the conditions we described the mitochondrial activities of complexes I, II, III and IV are stable for at least a year if the mitochondrial aliquots are stored at −70° C. We do caution that laboratories measuring enzyme activities on frozen muscle samples should generate their own range of normal activities for both fresh muscle and muscle which is frozen for the time period of the muscle sample they are investigating. We did not state that investigation of mitochondria from frozen muscle is as useful as from fresh, intact, muscle and the elegant data of Scholte et al. referred to by Drs. Scholte and Trijbels serve to emphasize that fresh muscle is the preferable sample for investigation of mitochondrial respiratory chain function.

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Third and fourth pouch/arch syndrome (Di George anomaly) in one dizygotic twin

Received: 10 May 1994
Accepted: 12 May 1994

Abbreviation *DGA* Di George anomaly

Sir: Third and fourth pouch/arch syndrome or Di George anomaly (DGA) is a rare developmental defect of the structures derived from the third and fourth (and partially first, second and sixth) pharyngeal pouches [1] with moderate to severe hypoplasia of the parathyroid glands and thymus, abnormal location of the thymus, malformations of heart, eye, mouth and ear. Clinical features include unusual facies, hypocalcaemic tetany, congenital conotruncal heart disease and increased susceptibility to viral, mycotic and bacterial infections. Immunological abnormalities vary in accordance with the faulty development of thymic epithelium. Very low numbers of T-lymphocytes and abnormal B-lymphocyte immunity are observed in complete thymic aplasia. The present is the first report describing DGA in one of two dizygotic twins.

The patient was born at 37 weeks of gestation by caesarean delivery; he weighed 1400 g. Facial features included micrognathia and low-set prominent ears. Oesophageal atresia was diagnosed. Echocardiography revealed tetralogy of Fallot. Calcaemia was below 8 g/dl. Lymphocyte subsets were determined at 5 days of life in peripheral blood mononuclear cells using fluorescent-activated cell-sorting after cell staining with monoclonal antibodies. Very low numbers of CD3- (T-lymphocytes = 234/mm^3), CD4- (helper-inducer T-cell subset = 174/mm^3) and CD8- (cytotoxic-suppressor T-cell subset = 42/mm^3) positive cells were found. Chromosome analysis, utilizing high-resolution banding techniques, detected no modifications. At age 6 days the patient died from cardiac failure. At post-mortem examination, the thymus was absent but small ectopic thymic lobes were detected beside the epiglottis.

At the time of writing the twin brother was 15 months old. His birth weight was 2600 g, no dysmorphism was observed and growth was normal. Calcaemia and circulating parathyroid hormone levels were normal. Clinical examination, electrocardiography and echocardiography showed no abnormalities. Chest X-ray showed a normally sized thymus shadow up to 3 months of life. T-cell and T-cell subset numbers, in vitro mitogen responsiveness and serum immunoglobulin levels were normal. Chromosome analysis showed no modification.

Family history was negative and parents were nonconsanguineous. They were clinically healthy and personal histories revealed no significant findings. Calcaemia, circulating parathyroid hormone levels and immunological findings were normal. Maternal clinical history during pregnancy was negative; drug and alcohol use were denied.

Findings in humans [3] and models in mice and chick embryos [1, 2] suggest that DGA depends on the failure of neural-crest mesenchymal derivatives to migrate and interact properly with pharyngeal epithelium. Causes of this failure are poorly understood. Familial occurrence (without a single mode of inheritance) has been reported, but most DGA are sporadic [1]. Thus, either environmental factors or genetic disorders negatively influencing embryogenesis have been suspected. Maternal diabetes or alcoholism have been associated with DGA and a condition resembling DGA is observed in newborn mice whose mothers were deprived of zinc or treated during early gestation with alcohol or isotretinoin [1, 6, 7]. Both teratogens affect cephalic neural crest development [6, 7]. On the other hand, deletions at 22q11 and, more rarely, at 10 p, 18q, 5p13 or 17p13 and isochromosome 18q have been observed in about 25% of cases [4, 8].

Explanation of our observation remains only a matter of speculation. We cannot rule out that a different degree of exposure to maternal blood (route of environmental factors) or a different susceptibility to environmental factors (due to inherent resistance or lack of favouring mechanisms) occurred in one twin. However, the effects of extrinsic factors are usually similar in both identical and dizygotic twins [5]. On the other hand, even though neither twin had evident chromosome alterations, DNA microdeletions (known to occur in DGA patients [4]) could be present in the affected twin. Thus, DGA in only one dizygotic twin would be more easily explained by intrinsic rather than extrinsic causes.

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