49 ESTIMATION OF PYRUVATE DEHYDROGENASE (E, ) ACTIVITY IN HUMAN SKELETAL MUSCLE.

HUMAN SKELETAL MUSCLE.
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The pyrivate dehydrogenase complex plays a central role in the

which pyruvate oxidation. The complex consists of five components of which pyruvate dehydrogenase ( $E_1$ ) catalyzes the rate limiting step.

step. Since  $E_1$  has a low activity in human skeletal muscle tissue a sensitive method is needed for diagnostic purposes. Measurement of  $^{12}CO_2$ -production from  $[1-^{12}C]$  pyruvate provides a specific and sensitive assay for measuring  $E_1$  activity in crude extracts. In the  $E_1$  assay an artificial electronacceptor has to be added. We use dichlorophenolindophenol (DCPIP) instead of the often are inhibitory affects on often applied ferricyanide. DCPIP shows no inhibitory effects on E, activity. E, activity appears to be dependent on the cofactors thiamineoyrophosphate and magnesium and is completely inhibited by fluoropyruvate. The method can be applied to frozen or fresh We established a difference in  $E_1$  activity between 600 g supernatant and homogenate of skeletal muscle tissue. The measurement of

tant and homogenate of skeletal muscle tissue. The measurement of E<sub>1</sub> activity in supernatant from frozen muscle appeared to be un-reliable. Control values in homogenate, 515-2080 nmol.hr<sup>-1</sup>.g<sup>-1</sup>. muscle, are higher or the same as those reported by others. With the new assay we identified two patients with a E<sub>1</sub> deficient

The method appears also suitable for measurement of  $\boldsymbol{E}_1$  activity in chorionic villi.

ANTENATAL DIAGNOSIS OF MITOCHONDRIOPATHIES. 50

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Various abnormalities in mitochondrial enzyme activities have been found, e.g. in myopathies. In only a restricted number of patients these disturbances can be demonstrated in fibroblasts. This restriction is caused by two facts: <u>1</u> some defects, found in muscle tissue, are not expressed in cultured fibroblasts and <u>2</u> some mitochondrial enzyme activities are hardly measurable in fibroblasts.

Recently, chorionic villi have become important for detecting aberrations in, among others, enzyme activities. Using chorionic villi instead of amnion cells fixes the moment of antenatal diagnosis at an earlier time. In the case of mitochondrial myopathies it is furthermore important that some enzyme activities can be measured in chorionic villi which can not be determined in fibroblasts. Among them are various oxidoreductases of the respiratory chain. Activity of cytochrome oxidase,pyruvate dehydrogenase complex and citrate synthase can be determined in both chorionic villi and fibroblasts.

Control values (in mU/mg protein; mean+ SD) in chorionic villi:

citrate synthase 49  $\pm$  18 NADH : O<sub>2</sub> oxidoreduct. 5.4  $\pm$  3.1 cytochrome oxidase 50  $\pm$  18 NADH : Q<sub>1</sub><sup>2</sup> oxidoreduct. 9.1  $\pm$  5.5 pyruvate DHcomplex 2.9  $\pm$  1.3 succinate : cyt c oxred 4.5  $\pm$  1.5 The possibilities for antenatal diagnosis of mitochondrial

myopathies have now clearly been extended.

CLINICAL HETEROGENEITY IN GAUCHER DISEASE TYPE 3. Maaswinkel-Mooy PD Poorthuis BJHM Van de Kamp JJP. Department of Pediatrics, State University Leiden, The Netherlands.

Gaucher disease (GD) is an autosomal recessive disorder of glycosphingolypid metabolism, resulting from the reduced activity of the lysosomal enzyme (3 -glucocerebrosidase. Three distinct phenothe lysosomal enzyme is -glucocereorosidase. Infee distinct pieno-types have been delineated, based on the absence or presence and severity of neuropathic involvement: type 1 GD or nonneuronopathic; type 2 or acute neuronopathic, rapidly fatal and type 3 or chronic neuronopathic. The three types must be due to different allelic Nutations since no complementation was found after cellfusion. Nevertheless a few publications have appeared describing different types of GD within the same family. Here we report genealogical and clinical data of three patients in one family, two brothers and their cousin, with a wide variation in clinical presentation. The two brothers had severe neurological symptoms from the first year, which place them in type 3; the cousin had no neurological abnormalities and clinically would have been classified as type 1. By immunoblotting techniques of fibroblast homogenates all three were classified as type 3 (or type 2). Our data show that extreme variation in clinical presentation is possible within the same biochemical phenotype, even within one family, which makes classification on clinical grounds alone unreliable.

EXCESSIVE GLYCEROL EXCRETION IN A MALE INFANT WITH PSYCHOMOTOR RETARDATION; ADRENOCORTICAL INSUFFICIENCY AND EARLY DEATH Søvik,O.,Jellum,E.& Madsen,B. Department of Pediatrics,University of Bergen and Institute of Clinical Chemistry, University of Oslo,Norway 52

A male, newborn infant(3480g,54cm) presented with vomit-ing, slightly dysmorphic facial features, and serum eling, slightly dysmorphic facial features, and serum el-ectrolytes compatible with adrenocortical insufficien-cy. Although the vomiting disappeared after hormone re-placement, the infant failed to thrive.Psychomotor re-tardation was noted, and he gradually developed spasti-city.There was "diffuse white matter disease" by cere-bral computer tomography.Chromosome analysis showed normal karyotype.Metabolic screening at 6 months of age revealed by sugar chromatography a marked spot with Rf-value 1.5 compared with glucose.By gas chrom-atography/mass spectrometry this substance was identi-fied as glycerol.The glycerol excretion(1.0-1.5g/2th) was unrelated to diet or drugs.The patient died 12 months old,during a respiratory tract infection.Fibro-blasts for glycerol kinase determination were not ob-tained. tained.

Glyceroluria, with or without demonstrated glycerol kinase deficiency, is an inborn error of metabolism of unknown incidence. The phenotypic expression is vari-able. Screening may easily be done by urine chromato-graphy(ethyl acetate: pyridine: H\_0;13:5:4) on paper or thin layer, and visualized by silver nitrate/acetone.



APPLICATION OF POLYMORPHIC CHROMOSOME 13-SPECIFIC PROBES TO LINKAGE STUDIES IN FAMILIES WITH HEREDITA-RY RETINOBLASTOMA

KY KEIINUBLASIUMA Scheffer (1), Tan KEWP (2), Buys CHCM (1) (1)Dept. Human Genetics, State Univ. Groningen, (2)Dept. Ophthalmology, State Univ. Utrecht The isolation of RFLP-revealing DNA sequences from band ql4 of chromosome 13 where the retinoblastoma locus has been mapped is chromosome 13 where the retinoblastoma locus has been mapped is a prerequisite for linkage studies in affected families. Until now we have isolated by various approaches nineteen unique chromosome 13-specific DNA sequences from fifteen different loci and subcloned these sequences into pBR329 or pUC9. To find out which sequences derived from 13q14 we constructed a mouse-human cell hybrid lacking 13q14 DNA by carrying out a somatic cell hybridisation between mouse RAG cells and fibroblasts from a hybridisation between mouse RAG cells and fibroblasts from a patient with retinoblastoma caused by a constitutional deletion of 13q14. We isolated a hybrid cell clone with the deleted chromosome but lacking the normal homologue. Six probes from three different loci did not show hybridisation with DNA from this cell clone. Their putative assignment to 13q14 could be confirmed by means of a mapping panel consisting of hybrid cell lines that we made monosomic for different parts of chromosome lines that we made monosomic for different parts of chromosome 13 by irradiation with X-rays. One of the 13q14 probes detects a low frequency MspI RFLP. A few probes located outside 13q14 revealed high frequency RFLPs. Two 13q14 probes isolated to date have been used to screen a total human cosmid library. Flanking unique human DNA sequences have been isolated from positive cosmids and a search for RFLPs is in progress. Isolated polymor-phic probes are now being applied to linkage studies in families with hereditary retinoblastoma.

LOCALIZATION BY IN SITU HYBRIDIZATION OF DNA PROBES WITH TIGHT LINKAGE TO THE LOCUS FOR THE CYSTIC 154 FIBROSIS MUTATION

PIBROSIS MUTATION van der Hout AH and Buys CHCM Department of Human Genetics, State University of Groningen, The Netherlands Probes 7C22 and pJ3.11 known to be closely linked to the cystic fibrosis locus were made available to us by Prof. Williamson, London. In situ hybridizations were carried out on metaphase preparations of lymphocytes from a patient having an apparent deletion in band q22 of one of the chromosomes 7, but no CF. The site and number of grains were scored for the normal and the deleted homologue. The majority of the grains resulting from hybridization with 7022 were found in the region q22-qter, with a cluster-ing at the distal part of band 31 and at band 32. For pJ3.11 most of the grains were in the region q31-qter. The normal homologue was more frequently labelled than the deleted one. This differ-ence might be due to random variation, although alternatively structural chromosome aberrations other than deletion might be

involved in this patient. In summary, our preliminary results suggest the distal half of the long arm of chromosome 7 to be the location of these probes. Our studies are now being extended to some other probes linked to the cystic fibrosis locus.