Propionyl-CoA carboxylase deficiency. A study on cellgenetic, biochemical, and clinical heterogeneity
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SUMMARY

Propionic acidemia, an inborn error of metabolism, is caused by a deficiency of propionyl-CoA carboxylase (PCC; E.C.6.4.1.3.), a mitochondrial matrix enzyme which catalyzes the biotin-dependent carboxylation of propionyl-CoA to form D-methylmalonyl-CoA. Clinical heterogeneity is present in propionic acidemia, but has failed to correlate with the residual enzyme activity. Cell genetic heterogeneity has recently been reported, but also failed to correlate with clinical heterogeneity. Biochemical characterization of the mutant enzyme has revealed that structural gene mutations are involved in PCC deficiency. Differences in biochemical properties seemed to correlate with cell genetic heterogeneity.

This thesis deals with certain clinical, cell genetic, and biochemical aspects of propionic acidemia. Moreover, some aspects concerning the origin of viable amniotic fluid cells as obtained in the second trimester of pregnancy, and the influence of culture conditions on the activity of PCC in cultured fibroblasts and amniotic fluid cells have been investigated.

Chapter II describes an attempt made to establish the origin of viable amniotic fluid cells as obtained before midpregnancy by transabdominal amniocentesis. For that purpose cells were collected from the oral cavity and vaginal vestibule of healthy newborns, and from the urine of healthy newborn males. These cells were brought into culture and the morphology of the cultured cells was compared with that of cultured second trimester amniotic fluid cells. Only pregnancies involving a normal fetus were included. It was concluded from the results that cells derived from the oral cavity and vaginal vestibule of healthy newborns cannot be cultured. Whether this is also true before midpregnancy is not certain. In contrast cells derived from the urinary tract of healthy newborn males can be cultured. The morphological similarities between these cultured cells and cultured second trimester amniotic fluid cells make it likely that the fetal urinary tract already contributes to the cell population which can be cultured before midpregnancy.

However, origins of viable amniotic fluid cells obtained in these cultured fibroblast-like amniotic fluid cells during the late first trimester and during the second trimester have been investigated.

PCC activity was examined in these cultured fibroblast-like amniotic fluid cells. The influence of the culture conditions on the acid activity of PCC was examined. The influence of culture conditions on the acid activity of PCC was examined.

Chapter III describes some aspects concerning the origin of viable amniotic fluid cells as obtained before midpregnancy by transabdominal amniocentesis. For that purpose cells were collected from the oral cavity and vaginal vestibule of healthy newborns, and from the urine of healthy newborn males. Although PCC activity was examined in these cultured fibroblast-like amniotic fluid cells during these conditions, the pH during culture was independent of the growth medium in fibroblasts and in fibroblast-like cells. The number of cells is dependent on the conditions. The maximum growth medium increased. Although PCC activity was examined in these cultured fibroblast-like amniotic fluid cells during these conditions, the pH during culture was independent of the growth medium in fibroblasts and in fibroblast-like cells. The number of cells is dependent on the conditions. The maximum growth medium increased. Although PCC activity was examined in these cultured fibroblast-like amniotic fluid cells during these conditions, the pH during culture was independent of the growth medium in fibroblasts and in fibroblast-like cells. The number of cells is dependent on the conditions. Therefore propioni...
However, origins distinct from the urinary tract may exist in this period of gestation.

Chapter III describes the general principles of fibroblast and amniotic fluid cell culture and the determination of PCC activity in these cultured cell types. The influence of the growth phase, and the influence of the pH of the growth medium on PCC activity were examined.

PCC activity varied with the growth curve of fibroblasts and fibroblast-like amniotic fluid cells. After trypsinization and replating of the cells PCC decreased during the lag phase, increased during the late logarithmic growth phase, and remained constant during the stationary phase of cultured fibroblast-like amniotic fluid cells. These variations, although not significant, were found consistent with the role of PCC in the catabolism of amino acids.

Maximum growth rate and cell density for fibroblast-like amniotic fluid cells were obtained between pH 7.0 and 7.4. A low pH during culture tended to increase PCC activity about two-fold in fibroblasts and fibroblast-like amniotic fluid cells, independent on the bicarbonate concentration. A small increase in PCC activity was observed at a higher bicarbonate concentration of the growth medium. Cell growth was markedly inhibited under these conditions. Above pH 7.6 both cell growth and PCC activity readily decreased. High concentrations of propionic acid in the growth medium increased PCC activity.

Although PCC activity appeared to be affected by the culture conditions, the effects found were too small to justify cell culture under special conditions. For prenatal diagnosis it is essential to choose such culture conditions that a maximum number of cells is obtained in the shortest possible time, with, at least for the controls, the highest possible PCC activity. Therefore propionic acid may be included in the growth medium.

The chapters IV - VII are dedicated to the inborn error of metabolism propionicacidaemia. Clinical heterogeneity as expressed
in patients with propionicacidemia, as well as cellgenetic and biochemical properties of PCC in fibroblasts of patients with a PCC deficiency were investigated in search for a possible correlation between the parameters studied.

Chapter IV deals with cellgenetic heterogeneity of propionicacidemia. Cellgenetic heterogeneity was demonstrated in 13 fibroblast lines derived from patients with PCC deficiency. In vitro biotin responsiveness had been excluded before in 11 of these cell lines and considered unlikely in the remaining two. Genetic complementation analysis was carried out in polyethylene glycol-dimethylsulfoxide induced heterokaryons constructed in monolayers of mixed mutant cell lines. PCC activity as reflected by incorporation of $^{14}\text{C}$-propionate into trichloroacetic acid insoluble cellular macromolecules was measured. By employing a double-label technique a correction could be made for differences in the rate of protein synthesis present in the individual cell lines. Positive control fusions with an in vitro vitamin $\text{B}_{12}$ unresponsive methylmalonyl-CoA mutase deficient cell line, as well as negative control self fusions of PCC mutant cell lines were carried out additionally to exclude false negative results. The usefulness of the double-label technique was evident in case of doubtful increase of $^{14}\text{C}$-propionate incorporation.

The existence of two distinct genetic complementation groups was demonstrated, indicating intergenic complementation is involved in in vitro biotin unresponsive PCC deficiency. No indications for intragenic complementation were found. The results are in agreement with the recessive mode of inheritance of the disease. The two structural genes involved could encode for the two non-identical subunits of which the native enzyme is composed, although other concepts are also possible.

Chapter V deals with kinetic properties of PCC in twelve of the same fibroblast lines studied in Chapter IV. In order to gain a better insight into the nature of the genetic defect, apparent $K_m$ and corresponding $V_{\text{max}}$ values for the substrates bicarbonate and propionyl-CoA were derived. The $K_m$ for NaHCO$_3$ and Eadie-Hofstee transformations were calculated for the cell lines, for which followed apparent first order conditions used.

The $K_m$ for NaHCO$_3$ carboxylation reaction of mutant cell lines the enzyme is impaired lines exhibited a lower reaction conditions site of PCC is also a genetic defect as the unlikely. Regarding in disagreement with deficiency. All these the same genetic complementation to encode for the case conditions affecting cell lines were discarded observed altered properties of PCC of one cell A defect localized in its catalytic activity enzyme was regarded as explaining were considered derived from sibling indicating that the subunit and affects line showed no abnormal lesion affecting of considered unlikely, genes encoding for PCC line could not be cl.
propionyl-CoA were determined from least square regression analysis of the kinetic data from both the Lineweaver-Burk double-reciprocal and Eadie-Hofstee transformed double-reciprocal plots. The mutant cell lines, for which kinetic studies could be carried out, followed apparent first order reaction kinetics under the reaction conditions used.

The $K_m$ for NaHCO$_3$, reflecting the functioning of the carboxylation reaction step of PCC was found increased in a number of mutant cell lines, indicating that the carboxylation site of the enzyme is impaired in these mutants. PCC of some of these cell lines exhibited a lowered $K_m$ for propionyl-CoA under the reaction conditions used, thus suggesting that the second catalytic site of PCC is also affected in these mutants. However, a double genetic defect as the cause of this complex kinetics was considered unlikely. Regarding these mutants as compounded heterozygotes is in disagreement with the current cellgenetic concept on PCC deficiency. All these mutant cell lines have appeared to belong to the same genetic complementation group of which the gene is supposed to encode for the carboxylation subunit of the enzyme. Reaction conditions affecting the results of the kinetic studies in these cell lines were discussed, and a kinetic explanation for the observed altered properties of PCC was found more logical.

PCC of one cell line was found to have a lowered $K_m$ for NaHCO$_3$. A defect localized in the carboxyl transferase subunit not affecting its catalytic activity, but changing the conformation of the enzyme was regarded as the most probable cause, although other explanations were considered possible too. PCC in two cell lines, derived from siblings, exhibited an increased $K_m$ for propionyl-CoA, indicating that the defect is localized on the carboxyl transferase subunit and affects the second catalytic site. PCC of one cell line showed no abnormalities of the kinetic parameters studied. A lesion affecting one of the catalytic sites of PCC was therefore considered unlikely, although a mutation in one of the structural genes encoding for PCC is still possible in this mutant. One cell line could not be classified on the basis of the kinetic properties
studied. Too low residual PCC activity precluded interpretation of the kinetic data.

The altered kinetic properties of PCC as found in ten of the twelve mutant cell lines infer that mutations in the structural genes encoding for the apoenzyme are involved in most of the PCC deficient cell lines studied. The implications of these altered kinetic properties of PCC for the disease state of the patients were discussed. Although lowered $K_m$ values do not seem to be of clinical importance, an increased $K_m$ for one of the substrates studied may have important consequences for the patient involved. An increased $K_m$ of PCC for NaHCO$_3$ implies a suboptimal functioning of the defective enzyme in catabolism.

Chapter VI deals with the case histories of the 13 patients, who suffered from propionic acidemia and whose fibroblast lines were investigated as described in Chapters IV and V. The cardinal features of the disease state, the course of the disease, and the most characteristic clinical-chemical findings in the blood and/or the urine of the patients were briefly summarized and commented. On the basis of these data the patients were classified according to the two forms of clinical expression as proposed by Hommes and De Groot (71): the acute neonatal form and the mild form. Seven patients were found to have the acute neonatal form, while six patients expressed the mild form. The difference in time of onset of the symptomatology was, in agreement with Hommes and De Groot (71), found to be the most rational criterion in the classification.

The acute neonatal form seemed to be more harmful, since five of the seven patients with this form of clinical expression died early in life. Additional symptoms like hepatomegaly, leucocytopenia (neutropenia), and thrombocytopenia were observed mainly in these patients, as was the elevation of the lysine concentration. The patients having the mild form of clinical expression seem to have a better chance of life. Osteoporosis appeared to be a characteristic finding in the older patients.

In Chapter VII the clinical characteristics of the three previous possible correlation to the literature clinical expression was to be present (at least provided that the results to be considered. The patients of group BC expressed the residual PCC activity.

In contrast nearly all complementation group one. However, the analysis incomplete. Most patients group A suffered from death early in infancy of the two surviving possibly because of having has been kept under control.

The altered kinetic properties of mutant cell lines subject of mutation in the state of PCC, and may in part the course of the disease

The current therapy propionic acidemia, as well as the second offspring in a

The results of this study clinical practice. First and second a refinement the justified decision for affected second offspring.
In Chapter VII the cellgenetic, biochemical, and clinical characteristics of the patients with propionic acidemia as described in the three preceding chapters are reviewed in search for a possible correlation. It is concluded that propionic acidemia is to be considered as a heterogeneous disease entity, in which in contrast to the literature cited a correlation between the clinical expression form and the genetic complementation group seems to be present (at least for the patients included in this study), provided that the residual PCC activity is also taken into consideration. The patients belonging to genetic complementation group BC expressed the mild form of the disease, except one, but his residual PCC activity appeared to be extremely low. In contrast nearly all the patients belonging to genetic complementation group A expressed the acute neonatal form, except one. However, the anamnestic data of this patient may have been incomplete. Most patients belonging to genetic complementation group A suffered from severe illness soon after birth, resulting in death early in infancy in five of these seven patients. However, of the two surviving patients, one has developed quite normal, possibly because of her high residual PCC activity. Moreover she has been kept under close medical supervision from birth on. The altered kinetic properties of PCC as found in most of the mutant cell lines substantiate the existence of different types of mutation in the structural genes encoding for the apoenzyme of PCC, and may in part have contributed to the severity and course of the disease in the patients involved.

The current therapeutic approaches for patients with propionic acidemia, as well as the possibility to prevent affected second offspring in a family at risk have been mentioned briefly. The results of this study may have two possible consequences for clinical practice. First a more adequate therapy of affected infants and second a refinement of prenatal diagnosis, leading to a more justified decision for interruption of pregnancy in case of affected second offspring.