SUMMARY.

The investigations described in this thesis are a contribution to the study of Leigh's disease (Subacute Necrotizing Encephalomyelopathy, SNE). SNE resembles in neuropathology Wernicke's encephalopathy, which is caused by thiamine deficiency.

The scope and the purpose of the present study is given in the introduction (chapter 1). From a review of the selected literature, given in chapter 2, it will be clear that the pathogenesis of Leigh's disease is far from understood.

The experimental procedures used in the investigations described, are outlined in chapter 3. The investigations focus on the following aspects:
- 1- a synthesis of thiamine triphosphate in vitro (chapter 4),
- 2- biotin deficiency in the newborn rat as an animal model for reduced pyruvate carboxylase activity in man (chapter 5 and 6),
- 3- the effect of thiamine on carbohydrate metabolism of normal rats and rats which show lactic acidosis as a result of biotin deficiency (chapter 7).

It has been reported that SNE brain is deficient in thiamine triphosphate, resulting from an inhibited synthesis of this phosphate ester of thiamine. Assay methods have been reported in the literature to demonstrate a synthesis of thiamine triphosphate in vitro. An inhibition of this synthesis by a certain glycoprotein, present in the body fluids of SNE patients was observed. A reinvestigation of these assay methods was undertaken and the efforts to demonstrate an in vitro synthesis of thiamine triphosphate are described in chapter 4. The results of this study indicate that the assays reported to demonstrate a synthesis of thiamine triphosphate in vitro are not reliable, because no net synthesis of thiamine triphosphate could be observed.

As a deficiency of pyruvate carboxylase has been reported in Leigh's disease, biotin deficiency in newborn rats was chosen as an animal model for reduced activity of this enzyme in man. A detailed description of the assay of pyruvate carboxylase is given in chapter 5. It was found that several precautions have to be made for the storage of tissue samples, the preparation of the tissue homogenate and the assay method, in order to demonstrate a reliable activity of this enzyme in human and rat tissues. The induction of biotin deficiency in newborn rats is described in chapter 6. The development of the pyruvate carboxylase activity of liver and of various parts of the brain was investigated in control and in biotin deficient newborn rats. While in control newborn rats a remarkable and steep increase of the activity of the liver enzyme was observed just after birth, the activity of this enzyme was extremely low in the neonatal period of biotin deficient rats. The decrease in the brain pyruvate carboxylase activity as a result of the biotin deficiency, was not as marked as found in liver. The highest pyruvate
carboxylase activity was observed in the brain stem. Although the activity of all biotin dependent enzymes is strongly depressed by biotin deficiency, this vitamin deficiency seemed to have resulted in a disease status for which most of the metabolic alterations investigated can be explained by a decreased activity of pyruvate carboxylase.

In Leigh patients, severe lesions in the brain, particularly in the brain stem, are observed at post-mortem investigations. Biotin deficiency induced in newborn rats did not evoke such lesions.

Results described in chapters 5 and 6 indicate that the pathogenesis of Leigh's disease cannot be explained by a deficiency of pyruvate carboxylase only.

It has been reported that administration of thiamine in pharmacological doses is beneficial to patients with lactic acidosis. As biotin deficient rats also show lactic acidosis, the results of a thiamine treatment of these rats were investigated and compared to the results obtained with control rats (chapter 7). Injection of high amounts of thiamine in the rat resulted in an increased concentration of liver mitochondrial thiamine diphosphate. Thiamine content of brain was not changed by thiamine treatment of the intact rat. This increase was accompanied by an increased excretion of lactic acid in urine and a lowered blood lactic acid concentration.

Alterations in the production and excretion of ketone bodies were also observed. The effect of thiamine on gluconeogenesis from lactic acid was investigated with isolated rat liver cells. It is suggested that administration of thiamine together with glucose and probably also aspartic acid, may have benefit to patients suffering from lactic acidosis, keto-acidosis and hypoglycemia, resulting from a defect in gluconeogenesis.

A general discussion of the results of the investigations described in this thesis is given in chapter 8.