Biochemical Basis of Thyroid Stimulation and Thyroid Hormone Action

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Contents

The Effect of Thyrotoxicosis on the Energy Providing Metabolism

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The most striking symptom of thyrotoxicosis is the stimulated basal metabolic rate. A warm skin, raised heart rate and fever indicate an enhanced metabolic rate and over-production of heat.

One of the processes markedly disturbed is oxydative phosphorylation. All measurements in thyrotoxic tissues found a stimulated oxygen consumption, decreased P/O-ratios and decreased ATP-levels (5, 8). The energy efficiency of the oxydative phosphorylation - normally 40-60 % - is strikingly reduced.

Some metabolic pathways are involved in stimulated respiration. The most important reducing equivalents for the reduction of oxygen in the respiratory chain of mitochondria are the pyridin-nucleotides and flavincoenzymes. However, a problem exists in that the mitochondrial membranes act as a permeability barrier to extramitochondrially produced hydrogen transferred to pyridinnucleotides: the mitochondrial membrane is impermeable to NADH. Bücher and Klingenberg (2) have postulated the glycerol phosphate shuttle in which the cytoplasmic NADH first reacts with cytoplasmic dihydroxyacetone phosphate, one of the intermediates of glycolysis, to form L-glycerol 3-phosphate. The glycerol 3-phosphate so formed readely penetrates through the mitochondrial membrane. Within the mitochondria the flavin-linked glycerol 3-phosphate dehydrogenase oxidizes glycerol 3-phosphate back to dehydroxyacetonephosphate, and its flavin prosthetic group becomes reduced. The reduced flavoprotein now donates its reducing equivalents to the electron-transport chain. In contrast to the oxidation of NADH, the oxidation of flavocoenzymes causes the oxidative phosphorylation of only two molecules of ADP. The energy yield is diminished by 30 %.

One of the most remarkable findings of experimental thyrotoxicosis is the sharp increase in the activity of mitochondrial glycerol phosphate dehydrogenase (6). The increase of the activity of glycerol phosphate dehydrogenase and of the flux rate of the shuttle occur in all tissues with increased respiration such as liver, heart, muscle and kidney. It seemed reasonable to consider the shuttle as a means by which thyroid hormones might influence metabolism.
However, for the thyrotoxicosis of man this statement is not relevant: in the thyrotoxicosis of man the activity of glycerol 3-phosphate dehydrogenase remains unchanged (7, 8, 9, 10, 11). In man other hydrogen transfer shuttles may be important, such as the aspartate-malate shuttle. Malate operates

as a reducing equivalent which is oxidized to oxaloacetate.

Further metabolic pathways are also involved in the increased turnover of substrates. In liver and skeletal muscle the glycogen content is reduced. In the EM-picture of the liver of a thyrotoxic patient (Fig. 1) only some few glycogen particles are stained in the cytoplasm. The atypical deposition of glycogen (gly) in the nucleus is noteworthy.

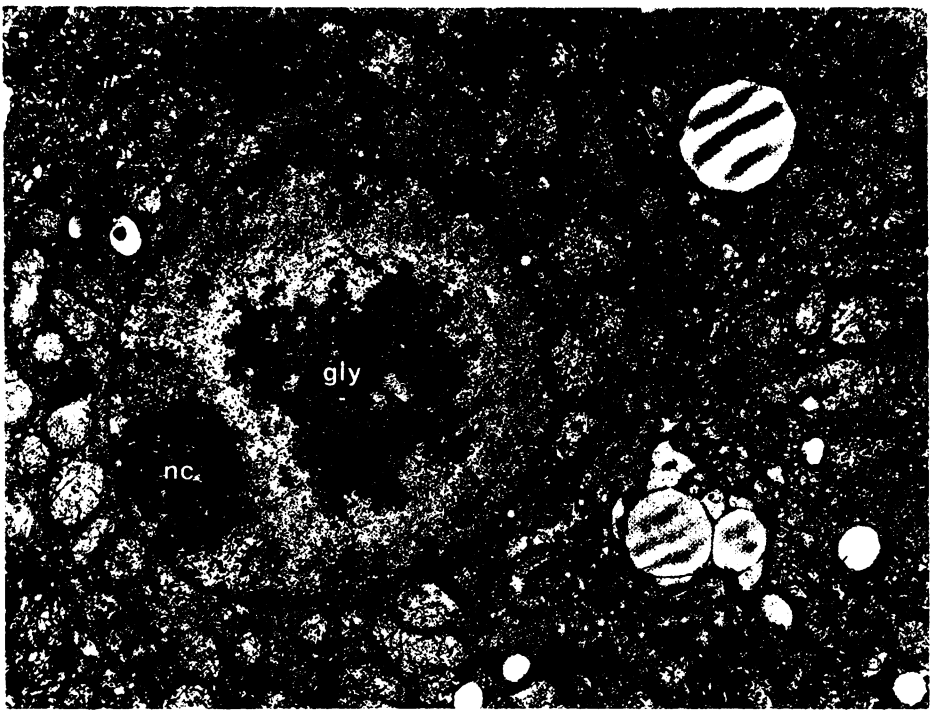

Figure 1: Liver of a thyrotoxic patient. Atypical deposition of glycogen (gly) within the nucleus (nc) nucleolus. Uranylacetate, lead citrate, x 5.000 (courtesy of W. Vogell, Fachbereich Biologie, Universität Konstanz).

The glycogen concentration is regulated by two cytoplasmic enzymes: glucogensynthetase and phosphorylase (Fig. 2). The activity of the two enzymes is sensitively regulated by hormones and metabolites. However, it is probable that in thyrotoxicosis, in particular the lysosomes are involved in the degradation of glycogen. This finding concerns the activity of 1,4-glucosidase (8).

Together with fatty acids, glycogen is the most important fuel for the muscle cell. The depletion of glycogen may cause the muscular weakness always claimed by the thyrotoxic patient. The entrance of glucose into the muscle cell is an energy linked step and coupled with the phosphorylation of glucose by

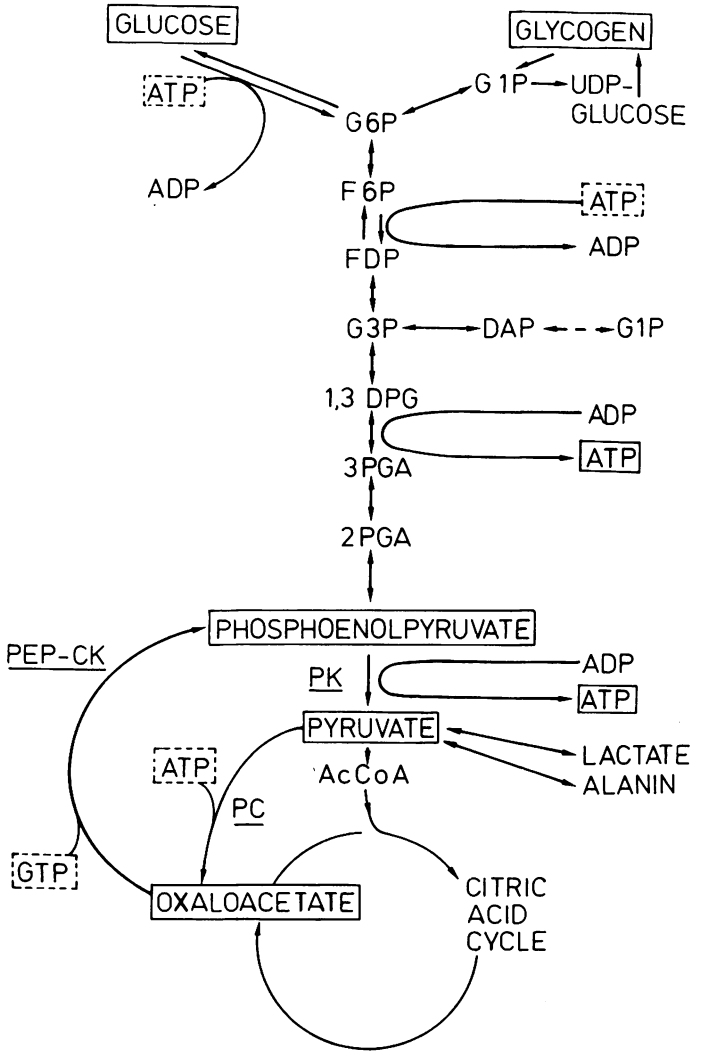

Figure 2: Pathway of glycogen metabolism, glycolysis, gluconeogenesis and citric acid cycle.

the hexokinase reaction.

In the skeletal muscle of thyrotoxic patients the acitivity of hexokinase is nearly doubled (7, 8), possibly as an adaptation to the lack of fuel. As can be seen by electrophoretic studies (7, 8) the increase of the hexokinase activity is restricted to isoenzyme II. For the muscle cell this method of energy supply remains critical, since in the acute case of need per Mol glucose 1 Mol ATP is consumed (Fig. 2). The phosphorylytic breakdown of glycogen, on the other hand, provides a phosphorylated hexose, glucose 1-phosphate. Because

of the high total weight of muscle, an increased carbohydrate catabolism could contribute considerably to the metabolism of the whole organism and might cause a significant increase in the basal metabolic rate.

A further symptom of the disturbed carbohydrate metabolism, found by the clinician, is the diabetic metabolism. For this several reasons are responsible, two of these will be discussed.

Together with skeletal muscle the liver is the most important organ in the regulation of blood glucose levels. After entry into the liver cell, glucose is immediately phosphorylated. This occurs by the hexokinase isoenzyme IV, glucokinase. The affinity to D-glucose is 2000 times lower than of the other hexokinase isoenzymes. In other words, when the hexokinase isoenzymes I-III show full activity, glucokinase has only reached 25 % of its maximal velocity. For this reason, the enzyme is suitable to regulate at blood glucose concentrations.

During artificial feeding of patients, it is well known that 0.4 g glucose per kg body weight are metabolized in 1 hour without the development of a hyperglycaemia. This corresponds to 28 g glucose per hour. Correspondingly, the phosphorylating capacity of the glucokinase enzyme is 20-24 g per hour (8). In thyrotoxicosis the activity of glucokinase is strongly diminished (7, 8). The specific staining of an electropherogram from a human thyrotoxic liver shows no activity of glucokinase when compared with the liver of an euthyroid control. In many examples it could be demonstrated that a normal glucose tolerance is accompanied by normal glucokinase activity, and that decreased glucose tolerance is accompanied by decreased glucokinase acitivity. The influence of hormones on the activity of the enzyme has consequences for the utilization of glucose.

The increased gluconeogenesis is the second method by which the hyperglycaemia in thyrotoxicosis is caused. Two limiting steps of the gluconeogenesis are catalyzed by pyruvate carboxylase (Fig. 2, PC) and phosphoenolpyruvate carboxykinase (PEP-CK). The activities of the two enzymes are increased by thyrotoxicosis, expecially in liver and kidney (7, 8).

The two reactions serve to synthesize phosphoenolpyruvate (PEP) from pyruvate (Py) (Fig. 3). This reaction obviously cannot occur by direct reversal of the pyruvate kinase reaction (reaction 1) because of its thermodynamical irreversibility. The overall reactionsof pyruvate carboxylase and phosphoenolpyruvate carboxykinase (reaction 2 and 3) show two high-energy phosphate bounds of ATP or NTP which are ultimately consumed to bring about the formation of a single PEP. In reality the energy costs are much higher: the freshly and expensively synthesized PEP is partially remetabolized back to pyruvate by the pyruvate kinase reaction (Fig. 4). In thyrotoxicosis and some other metabolic disorders this circulation can be strikingly increased. In this case the expression "futile cycle" characterizes well the metabolic situation (12, 8). The heat production of such energy traps is considerable and reduces the P/O-ratio. Energy in the form

$$(1)\quad PEP + ADP \xrightarrow{PK} Py + ATP \qquad -7.5\ Kcal$$

$$(2)\quad Py + ATP + CO_2 \xrightarrow{PC} OxAc + ADP$$

$$(3)\quad OxAc + GTP \xrightarrow{PEP\text{-}CK} PEP + GDP + CO_2$$

$$(2,3)\quad Py + \boxed{2\ NTP} \longrightarrow PEP + 2\ NDP$$

Figure 3: The reversal of the pyruvate kinase reaction (PK) (reaction 1) by the pyrovate carboxylase (PC) (reaction 2) and phosphoenolpyruvate carboxykinase (PEP-CK) (reaction 3). The overall reactions (2 and 3) show 2 high-energy phosphate bounds of ATP or NTP which are consumed to bring the formation of a single PEP.

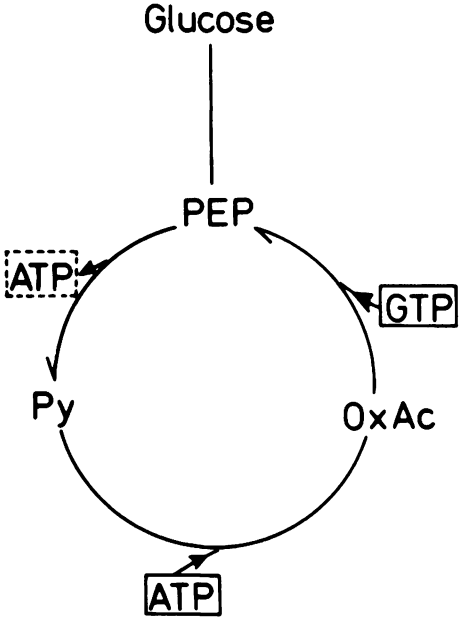

Figure 4:

The formation of phosphoenolpyruvate (PEP) from pyruvate (Py) and the remetabolization back to pyruvate as a "futile cycle".

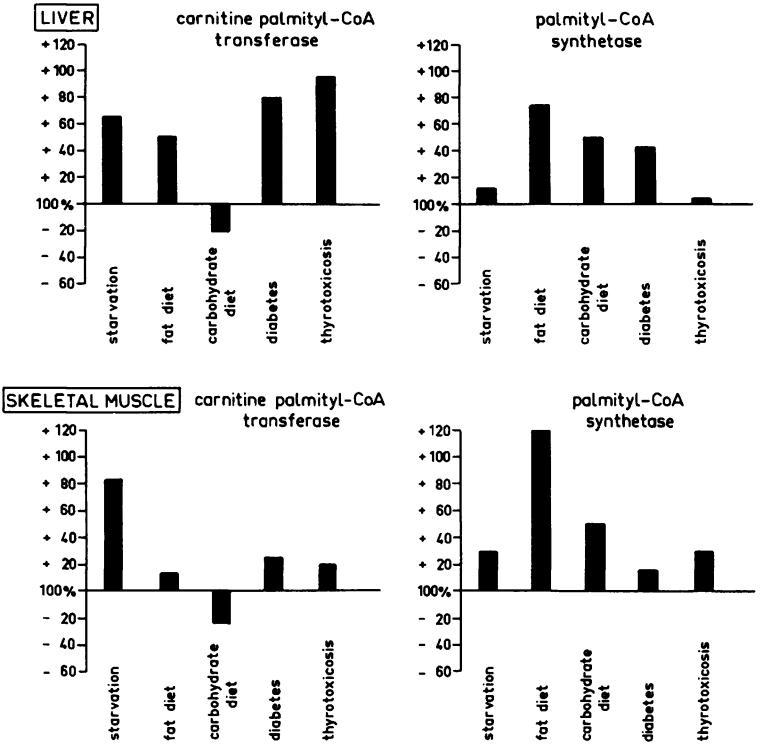

Figure 5: The activities of carnitine palmityl-CoA transferase and palmityl-CoA synthetase in rat liver and skeletal muscle under the influence of starvation (3 days), fat diet (8 days), carbohydrate diet (8 days), experimental diabetes (streptozotocin, 70 mg/kg, 6 days after injection) and thyrotoxicosis (15 µg 3,3',5-triiodo-L-thyronine/100 g body weight for 8 days) as compared with controls (100 % level). The values are given as means of 10 animals per group.

of heat is lost for the organism, the cell utilizes chemical energy only.

The respiratory quotient was the first indication of a stimulated turnover and oxidation of fatty acids in thyrotoxicosis. This corresponds to the finding that two rate limiting enzymes for the oxidation of fatty acids are induced in organs with the equipment for an oxidative metabolism (1, 8). We have investigated the carnitine palmityl-CoA transferase and palmityl-CoA synthetase in the liver and skeletal muscle of thyrotoxic rats (Fig. 5). The transferase activity is elevated in liver and muscle, the synthetase activity only in muscle. Whereas the synthetase activates long-chain fatty acids and yields palmityl-CoA, the special function of the carnitine palmityl-CoA transferase is to overcome the mitochondrial

barrier for palmityl-CoA, by transferring the fatty acid to carnitine. The finding of Fritz and Bremer (4, 3) showed that especially the carnitine palmityl-CoA transferase is found to be a rate limiting enzyme for the oxidation of fatty acids. The finding of Fritz was stimulated by the ability of carnitine to induce a maximal oxidation of fatty acids when it is added to isolated mitochondria. In thyrotoxicosis the enhanced turnover and oxidation of fatty acids is not able to overcome the energy crisis. The NADH produced by the ß-oxidation of fatty acids flows into a respiratory chain working at reduced efficiency.

In summary: In spite of a high flux rate of all oxidative processes, thyrotoxicosis is accompanied by a striking lack of ATP. The energy efficiency is reduced. In addition, there are ATP-traps described as futile cycles. The hemodynamic consequences are obvious: The oxidation of 1 Mol NADH with 1/2 Mol oxygen yields the energy to form 3 Mol ATP. A simple calculation shows that for this reaction 11.2 l oxygen are required, transported by 167 l of blood. This would require 40 min at a normal heart rate (8).

In thyrotoxicosis the futile increase of ATP-consumption and heat production is only possible by a critical raising of the cardiac output.

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Discussions

Chairman: H. BREUER

SEIF: In this very complicated context I would like to refer to a metabolic pathway where ATP is not wasted, but used. Very recently Liberman et al. (Endocrinology 97, 13, 1975) have shown that thyroid hormone induces an increased activity of the Na^+, K^+-dependent ATPase in cellular membranes. The number of enzyme units is increased by 30-40 %. They calculated that most of the additional heat production can be derived from this increased number of ATPase sites and their use of ATP. Where is it known from that the Bücher shuttle is not operating in the human with respect to thyrotoxicosis?

NOLTE: This is a result of our laboratory. It has been published in Eur. J. Clin. Invest. 2, 141, 1972.

SEIF: Where did you get the material from, by liver biopsy?

NOLTE: Yes, by liver punction.