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THE MECHANISM OF ACTION
OF THYROID HORMONES

C. Martius (Zürich, Switzerland)

Many hormones show marked physiological and clinical effects when in deficiency or excess, and yet may have only a small influence on the activity of enzymes. It has therefore not yet been possible to determine their biochemical point of attack. On the other hand the number of enzyme systems which are clearly influenced by the thyroid hormone is so great that here the problem is to localise the primary point of attack exactly and limit it from subsequent reactions. Thus for example the addition of thyroid hormones in vivo leads to an increase in the activity (as measured by the concentration in the tissues) of a whole series of enzymes which are vitally concerned in the combustion process of the cell. Examples are cytochromoxydase, cytochrome C, succinoxydase and others. Conversely thyroidectomy decreases their content. There appears at first to be a connection between these enzymes of respiration and the well known effect of thyroid hormone in increasing combustion. Nevertheless in all these cases the effect is doubtless only a secondary one. Thus the problem is, why in normal animals in a state of rest is the available capacity of the enzyme system for tissue respiration used to only a small extent, while under the same conditions in a hyperthyroid animal it can be used to the limit of its capacity? During the last decade biochemical investigations have provided the basis for understanding the principles of these hormone actions.

For understanding the effect of thyroxine, an observation of fundamental significance was that in the cell the magnitude of the cell respiration is automatically regulated by the energy consumption. The combustion of nutrient material in all cells is coupled with the generation of energy through a process which is termed oxydative phosphorylation, because in it energy-rich adenosine triphosphate (ATP) is formed from inorganic phosphate and adenosine diphosphate (ADP). This material is available to the cells for the various energy-consuming reactions, whereby it is decomposed back into inorganic phosphate and adenosine diphosphate. If the phosphate acceptor,
adenine diphosphate, is consumed by conversion into the triphosphate, there appears a limit on the cell respiration. The finer details of this are as little known at present as are those for the formation of the triphosphate during the combustion of the hydrogen of the nutrient material. The important point is that in the normal cell there exists a rather strong coupling between the energy-producing combustion processes and the energy-consuming cell processes. This coupling can be loosened or even completely broken by various interferences. It may occur, for example, by alteration of the structure of the respiring and phosphorylating cell particles, the mitochondria or sarcosoma. More important however, is interference by chemical substances, as was first shown with 2,4-dinitrophenol. This material, and an entire series of other substances since found, can loosen or completely sever the coupling between cell respiration and production of ATP. Then the combustion processes are no longer controlled by the production of ATP, and the energy of the combustion reaction is partially or completely liberated as heat instead of being bound in ATP. The molecular mechanism of this uncoupling is just as unknown as the entire enzymatic processes of oxydative phosphorylation.

It is clear that the known metabolic effect of the thyroid hormone can be readily explained on the basis of such an uncoupling of respiration and phosphorylation. It turns out that thyroxine also belongs to the group of materials that effect the uncoupling, and its effect can be demonstrated by application either in vivo or in vitro. For this it is necessary to carefully isolate the mitochondria and to determine their respiration and production of ATP by known methods. The action of sufficient quantities of thyroid hormone becomes evident in a lowering of the quotient of formed ATP to consumed oxygen (P/O ratio). The degree of lowering is determined to a certain extent by the quantity of hormone employed. That one is actually dealing with the decisive effect of the thyroid hormone in this case is made clear by the relation which exists between the influence of the basal metabolism and the oxydative phosphorylation measured on the same animal. It is easy to see that an impairment of the efficiency of the energy-producing processes by the hormone must lead positively and automatically to an increase of cell combustion - that is to the basal metabolism - since at least the same energy requirement has to be satisfied as for normal animals.

The two quantities can be connected by a simple mathematical expression so that one can calculate from the lowering of the P/O ratio the increase in the basal metabolism and vice versa. The calculated values agree in first approximation with those found, and this may be taken as evidence for the validity of the uncoupling theory for the thyroid hormone.

These experiments can then be extended to animals which have been made hypothyroid through thyroidectomy or by feeding with thiouracils. In these cases one finds the P/O ratio increased above normal, which agrees
with the lowering of the basal metabolism. In these cases one approaches the theoretical maximum values of 3 for the P/O ratio. In the normal animal this is lowered by thyroxine to about 2.2 - 2.4 and in a thyroid-poisoned animal it can be still lower (1.8 - 1.4 or less).

The question of the finer mechanism of the uncoupling effected by thyroxine cannot be answered at this time. The various substances which cause uncoupling, which belong in part to completely different classes of materials, probably also correspond to different possibilities for the reaction mechanism. In the case of thyroxine there appears to be an especially irregular uncoupling mechanism. If the treatment of mitochondria with thyroxine leads to an influence on the oxidative phosphorylation, it also produces a swelling of the mitochondria. This is not observed in the action of dinitrophenol. This leads one to think that an alteration of the permeability of the mitochondrial membrane by the hormone may be the cause of the lessened rate of phosphorylation. But whether these structural changes of the mitochondria must be regarded as a consequence of a disturbance of the enzyme reactions, or conversely as a primary structural alteration that disturbs the interplay of the enzyme system, cannot yet be decided.

Another unsolved problem is the question of the most active form of thyroid hormone. There is now, however, agreement that the hormone effect is due to thyroxine or related lower molecular materials, and not to any thyroxine-containing peptides or proteins.

In recent years a series of observations have been made which indicate that metabolic products of thyroxine can be formed in the body which have a greater influence on the basal metabolism and the oxidative phosphorylation than has thyroxine itself. Chief among these are substances resulting from deiodination, such as triiodothyronine. It seems completely general that substances with only 3 iodine atoms in the molecule are more rapidly and strongly effective than those with 4 iodine atoms. Compounds with modified side chains as tetra- or triiodothyroacetic acid are still more effective than thyroxine or triiodothyronine in some hormone tests (tadpole test), although with mammals this increase of activity is not found. In each case it must therefore be considered that the physiological and perhaps still more the pathological effect of the thyroid hormones is to be ascribed not only to the thyroxine but also - and perhaps predominantly - to these metabolic products. Perhaps there is even a connection between the formation of these substances in the cells and the known fact that the effect of thyroxine does not appear suddenly but after a certain delay.