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# On the Influence of Anti-thyroid Substances on the Hormone Production of the Thyroid Gland

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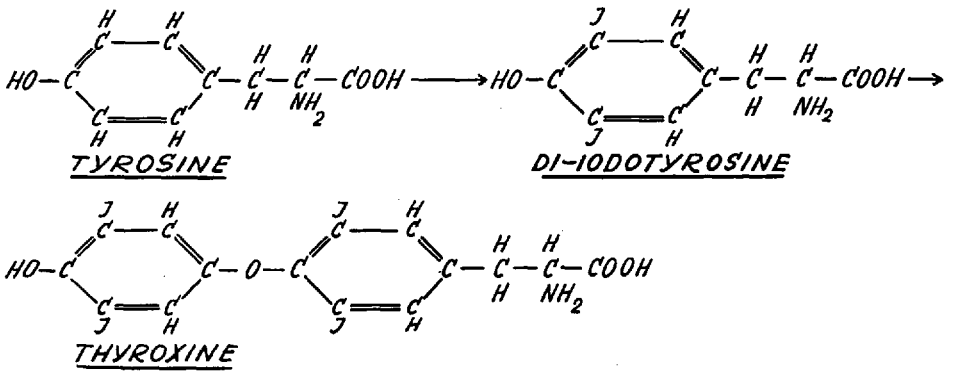
## 1. *Introduction*

During the last few years endocrinologists have taken more interest in the thyroid gland than some years ago. Before the second World War we had come to a deadlock, as the experimental possibilities of penetrating into the secrets of the thyroid gland were very limited.

The discovery of the thyroid-stimulating hormone (TSH) of the anterior pituitary had revealed only the relation: pituitary-thyroid and the morphological basis of the hormone-producing process in the thyroid gland, without disclosing anything of the essential events or of the rôle of TSH during this process.

Significant progress was made only in the analysis of the chemical constitution of the thyroid hormone (TH). It was found that thyroxine is the essential component of TH, originating after iodization of the amino-acid tyrosine, followed by condensation of two molecules of di-iodotyrosine. This is illustrated by the formula:




During and after the war very important advances were made with the help of two new experimental techniques. These not only led to the discovery of the essential processes of TH-formation, but also allowed the beginning of a broad inquiry into the function of TSH and its connection with TH. These two techniques were the use of radio-active iodine and the anti-thyroid substances. We shall occupy ourselves only with the last-named substances.

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In 1928 CHESNEY[1] *et al.* turned their attention to the phenomenon that rabbits after feeding on cabbage often exhibit a goitre, caused by a massive enlargement of the thyroid gland. Histological investigation of such thyroids showed that the number of cells and the vascularity had increased, but also that the colloid content of the follicles had decreased. Therefore there must be in the cabbage plant a "goitrogenic" substance (later on called "anti-thyroid" substance). As cabbage plants contain much organic cyanide, it was thought that these were responsible for the goitrogenic activity. SPENCE[2] found indeed that cyanides have a goitrogenic action.

A considerable time later various investigators (BARKER *et al.*[3], FAHLUND[4], RAWSON[5] *et al.*) were looking more in the direction of the thiocyanates. They observed that patients treated with thiocyanate for some other disease showed goitre symptoms. It was found that iodine and thyroid preparations have a prophylactic effect in relation to these symptoms.

At the same time a New Zealand research group was interested in the typical action of cabbage. KENNEDY and PURVES[6] studied the influence of seeds of several races of *Brassica* on the thyroid of rats, and observed the activation phenomena mentioned above. Moreover

[1] A. M. CHESNEY *et al.*, Bull. John Hopk. Hosp. *43*, 261 (1928).
[2] A. W. SPENCE, St. Barth. Hosp. Rep. *67*, 201 (1934).
[3] M. H. BARKER *et al.*, J. Amer. med. Ass. *117*, 1591 (1941).
[4] G. T. R. FAHLUND, Proc. Staff. Meet. Mayo Clin. *17*, 289 (1942).
[5] R. W. RAWSON *et al.*, Ann. Int. Med. *19*, 829 (1943).
[6] F. H. KENNEDY and H. D. PURVES, Brit. J. exper. Path. *22*, 241 (1941).

GRIESBACH[1] examined the anterior pituitary during treatment with that seed, from which he concluded that changes occur in the anterior pituitary closely related to the changes in the thyroid caused by goitrogenic factors in the seed.

After the fundamental investigations of GRIESBACH, KENNEDY and PURVES[2] it was possible to sum up the broad outlines of the interaction: pituitary-thyroid in connection with the goitrogenic influence of the *Brassica*-factor. They studied the influence of *Brassica* seed on the thyroids of hypophysectomized rats and found that these became inactive. They also observed that in thyroids of rats hypophysectomized after a long treatment with seed, histological activity rapidly decreased. So these investigators came to the modern conception of the antithyroid action: prevention of TH-synthesis → decrease of TH-concentration in blood → increase of TSH-production → histological activation of the thyroid gland.

KENNEDY[3] supposed that the goitrogenic *Brassica*-factor might be a thiourea-derivative, in which he was supported by his later discovery of the goitrogenic potency of thiourea and of allyl-thiourea. RICHTER and CLISBY[4] observed the same potency for phenyl-thiourea.

At the same time that BARKER discovered the goitrogenic potency of the thiocyanates and the New Zealand research group analysed the activity of the *Brassica*-factor and related it with the thiourea-derivatives, MACKENZIE, MACKENZIE and MCCOLLUM[5] found still another group of anti-thyroid substances; they studied the influence of sulfanilylguanidine on the metabolism of the gut flora of rats and incidentally observed that the thyroid glands of the experimental animals were 3–8 times as large as those of the controls, having also a higher activity. In the same publication they mentioned that they were busy studying other "sulfa drugs", as well as thiourea. This publication was followed by a stream of observations and experiments, causing an important increase of our knowledge of the physiology of the thyroid gland.

In recent publications, ASTWOOD[6] *et al.* and CARROLL[7] demonstrated that 1-5-vinyl-2-thio-oxazolidon is the typical *Brassica*-factor.

### 2. *The hormone production in the thyroid gland*

As stated above, thyroxine is the essential component of TH. It is linked to a protein with an albumin nature.

We must realize that in the formation of TH we are considering the following processes: concentration in the thyroid cells of iodides circulating in blood; liberation of iodine from the iodides, iodization of tyrosine in a tyrosine-containing protein with a globulin nature, condensation of di-iodotyrosine and thus formation of thyroxine, secretion of the so-formed thyreoglobulin into the lumina of the follicles, conversion of the thyreoglobulin to thyreoalbumin, and finally secretion of the thyreoalbumin into the bloodstream. In outline we can distinguish four stages in the process of the formation and release of thyreoalbumin:

(*a*) the action of the cells in concentrating iodides,
(*b*) the formation of thyreoglobulin,
(*c*) the accumulation of thyreoglobulin and formation of thyreoalbumin in the colloid,
(*d*) the delivery of thyreoalbumin by the cells.

Before we can discuss the specific actions of the antithyroid substances, we have to consider these processes.

(*a*) *The iodide-concentrating action.*—With the help of radio-active iodine it was possible to study this process exactly. VANDERLAAN and BISSELL[1] injected radio-iodide into animals and after a few minutes found it again in the thyroid. LEIN[2] observed organically bound radio-iodine already after 5 minutes (in the form of di-iodotyrosine or thyroxine), the maximum concentration of radio-iodides being found after 10 minutes. Gradually the concentrated inorganic iodine is transmuted into organic iodine. It is curious that the thyroid concentrates especially the elements of the seventh group of the periodical system: besides iodine also bromine, manganese and rhenium (BAUMANN *et al.*[3]) TSH has no influence on the iodide-concentrating process: KEATING[4] *et al.* observed thyroid hypertrophy 24 hours after TSH-injection, but an increased iodide-concentrating action only after 48 hours. As yet the quintessence of the iodide-concentrating process is unknown. It is thought by most authors (e. g. SALTER[5]) that in the thyroid a loose linkage with colloidal proteins takes place.

(*b*) *The formation of thyreoglobulin.*—Before the thyroid cell can begin with iodization of a globulin protein, it must have free iodine at its disposal. Thus there has to be a strong oxydizing system, capable of liberating iodine from the iodides. In this connection many authors thought of cytochrome-oxydase. The investigations of LERNER and CHAIKOFF[6] and of GLOCK[7] have established that this idea is incorrect. By studying e. g. the respiration of thyroid-tissue, and

[1] W. E. GRIESBACH *et al.*, Brit. J. exper. Path. *22*, 245 (1941); *22*, 249 (1941).
[2] W. E. GRIESBACH *et al.*, Brit. J. exper. Path. *22*, 245 (1941); *22*, 249 (1941). – F. H. KENNEDY and H. D. PURVES, Brit. J. exper. Path. *22*, 241 (1941).
[3] F. H. KENNEDY, Nature *150*, 233 (1942).
[4] C. P. RICHTER and K. E. CLISBY, Arch. Path. *33*, 46 (1942).
[5] C. J. MACKENZIE, J. B. MACKENZIE, and A. MCCOLLUM, Science *94*, 518 (1941).
[6] E. B. ASTWOOD *et al.*, Science *109*, 631 (1949).
[7] K. K. CARROLL, Proc. Soc. exper. Biol. Med. *71*, 622 (1949).

[1] W. P. VANDERLAAN and A. BISSELL, Endocrinol. *38*, 308 (1946).
[2] A. LEIN, Endocrinol. *32*, 429 (1943).
[3] E. J. BAUMANN *et al.*, Endocrinol. *33*, 44 (1944); Proc. Soc. exper. Biol. Med. *72*, 502 (1949).
[4] F. R. KEATING *et al.*, Endocrinol. *36*, 137 (1945).
[5] W. F. SALTER, Ann. Rev. Biochem. *14*, 561 (1945).
[6] S. R. LERNER and I. L. CHAIKOFF, Endocrinol. *37*, 362 (1945).
[7] G. E. GLOCK, Nature *158*, 169 (1946).

adding substances preventing up to 80% of the formation of di-iodotyrosine and of thyroxine (so if the cytochrome-oxydase plays a part in the hormone production, it must partly be occupied by these substances), it was found that the oxygen consumption was not influenced. Consequently the cytochrome-oxydase cannot be related to the production of thyroid hormone. By DEMPSEY[1] and other investigators peroxydase is held responsible for the reaction.

In general the processes of iodization and condensation also are attributed to enzymes. But a number of authors think that the hormone synthesis is a chemical process acting entirely without enzymes. They tend to quote the publication of LUDWIG and VON MUTZENBECHER[2], who could synthesize thyroxine by treating proteins with iodine at a special $p_H$. This opinion can be found e. g. in FORMIJNE[3]. As we shall see below, he connects the anti-thyroid action of the thiourea derivatives with this hypothesis; a theory against which we shall raise an objection.

(*c*) *Accumulation of thyreoglobulin and formation of thyreoalbumin.*—After producing thyreoglobulin, the cells secrete it into the lumen of the follicle, where it forms the colloid. Following thyreoglobulin they also secrete enzyme granules into the colloid. OKKELS[4] first described this. DE ROBERTIS[5] collected some colloid by means of micro-dissection. It was evident that it contained a proteolytic enzyme, as it could break down a gelatin substrate. So this enzyme probably reduces the size of the globulin molecules. The albumin molecules so formed are absorbed by the cells and afterwards secreted into the blood vessels.

The viscosity of the colloid is regulated by the action of a glycoprotein and a spreading factor also secreted by the cells into the colloid (GERSH[6], LEVINE[7]).

(*d*) *The delivery of thyreoalbumin.*—This is the single part of the process of the TH-formation which is directly influenced by TSH. The manner in which delivery is brought about has hardly yet been investigated. This is due to the slight knowledge we have of TSH. After TSH-injection the cell becomes higher and secretes granules containing proteolytic enzymes; the formation of thyreoalbumin increases, and thus more TH is delivered into the bloodstream.

### 3. *Influence of the anti-thyroid substances.*

After this concise review of the steps of hormone-synthesis in the thyroid gland, we can now discuss the actions of the anti-thyroid substances. It is found that these substances influence not one, but several parts of the hormone synthesis. We shall discuss them in succession.

(*a*) *Blockage of the iodide-concentrating action.*—Soon after the discovery of the anti-thyroid influence of the thiourea derivatives it was found that thyroids of animals treated with these substances show a decreased capacity to concentrate radio-iodides (FRANKLIN *et al.*[1], RAWSON[2] *et al.*). Therefore many investigators thought that the blockage of the iodide concentrating action was the single specific action of the thiourea-derivatives.

Later on (VANDERLAAN and BISSELL[3], VANDERLAAN and VANDERLAAN[4], TAUROG[5] *et al.*, ROMELL[6]), however, it was observed that these substances had no influence at all on the concentrating action. The differing results of the earlier authors can be explained by the fact, found afterwards, that the iodide-concentrating action takes place very soon after injection of the iodides (see above), through which the thyroid is rapidly saturated with iodides. In the normal case the iodides are brought into the iodization process and then, bound in the thyreoglobulin secreted into the lumen of the follicle, so that new iodides can be concentrated. As we shall see, the thiourea derivatives block the iodization process. Therefore during treatment with these substances the iodides are not transported, and thus there need to be no renewed iodide-concentrating action.

In contrast to the thiourea derivatives the thiocyannates have an influence merely on the concentration process and not on the iodization process (WOLFF *et al.*[7]).

It can easily be seen that the availability of such specifically acting substances is of great importance for endocrinological research.

(*b*) *Blockage of the iodization process.*—As was explained above, the thiourea derivatives act by preventing the iodization process. No thyreoglobulin and thus no active hormone is formed.

As GRASSO[8] observed, however, the thyroid cell, independent of the added substances, produces an inactive globulin product. In the colloid this product is broken down to albumins with the help of the uninfluenced proteolytic enzyme. These albumins will be secreted into the blood vessels. Thus hormone production continues, but the product is inactive. GRASSO[2] and DEMPSEY[9] thought there was a relation between the block and the peroxydase system, which is probably

[1] E. W. DEMPSEY, Endocrinol. *34*, 27 (1944).
[2] N. LUDWIG and P. v. MUTZENBECHER, Z. Physiol. Chem. *258*, 195 (1939).
[3] P. FORMIJNE, Proc. Kon. Akad. Wetensch. Amsterdam *49*, 484 (1946); Ned. Tijdschr. Geneeskunde *91*, 1800 (1947).
[4] H. OKKELS, C. r. Soc. Biol. *116*, 251 (1934).
[5] E. DE ROBERTIS, Western J. Surg., Obst. Gyn. *56*, 253 (1948).
[6] I. GERSH, J. End. *6*, 282 (1950).
[7] M. D. LEVINE, J. End. *6*, 288 (1950).
[1] A. L. FRANKLIN *et al.*, Endocrinol. *34*, 265 (1944).
[2] R. W. RAWSON *et al.*, Endocrinol. *34*, 245 (1944).
[3] W. P. VANDERLAAN and A. BISSELL, Endocrinol. *38*, 308 (1946).
[4] J. E. VANDERLAAN and W. P. VANDERLAAN, Endocrinol. *40*, 403 (1947).
[5] A. TAUROG *et al.*, J. Biol. Chem. *171*, 189 (1947).
[6] L. G. ROMELL, Schweiz. med. Wschr. *78*, 810 (1948).
[7] J. WOLFF *et al.*, Endocrinol. *39*, 140 (1946).
[8] R. GRASSO, Anat. Rec. *95*, 365 (1946).
[9] E. W. DEMPSEY, Endocrinol. *34*, 27 (1944).

responsible for the iodization. Most investigators are of the opinion that the block is correlated with an enzymatic process. Moreover, the hypothesis can be advanced against this that no enzymes are acting during hormone synthesis and blockage. FORMIJNE for example points to the following experiments *in vitro:* two solutions are made

(*a*) a solution of casein, bicarbonate and urea,

(*b*) a solution of casein, bicarbonate and thiourea.

After adding iodine to both solutions and waiting for some time, one can separate thyroxine out of the two solutions by extraction with butyl alcohol. In an actual experiment the first solution produced 32 mg, the second 3 mg.

WERNER[1] had showed already that iodine oxidizes the thiourea derivatives very easily. Whether one may reduce the processes in the living thyroid to such simple reactions or not forms a difficult problem. BAUMANN *et al.* made the following supposition: if the reaction described by WERNER takes place in the thyroid, it must be possible to prevent the goitre due to thiourea by giving a little more iodine than necessary to bind all the thiourea. They injected rabbits with thiourea and with a more than equivalent quantity of NaI every 4 hours over a period of 48 hours. Despite the iodide, symptoms of activation were observed. Therefore, according to BAUMANN, we must suppose that the thiourea derivatives act by blocking an enzyme system responsible for thyroxine formation.

(*c*) *Influence directly on TSH.*—RAWSON *et al.*[2] observed the following extremely important phenomenon in tissue cultures of thyroid: slices of normal rabbit thyroids were cultivated at 37°C in a tyrode-solution containing TSH. After 48 hours the analysed medium showed no TSH-activity at all. But after treating the inactivated medium with thiouracil or other anti-thyroid substances, the TSH-activity was shown to have been recovered. The anti-thyroid substances had no anti-thyroid influence in the concentrations used for reactivation of the TSH. Moreover, normal active hormone was shown to increase in stimulating capacity after treatment with anti-thyroid substances. This phenomenon is called hyperactivation. Therefore RAWSON *et al.* claim that anti-thyroid substances exercise an influence on the activity of TSH beside their action on the iodization process.

The investigations in this direction were continued by ALBERT[3] *et al.* Earlier it was stated that by treating TSH with elementary iodine most of the proteins of TSH were precipitated. The brown iodinated precipitate was shown to have lost 95–100% of its original activity. It was possible to remove 60% of the iodine with acetone, after which the rest again had its full TSH-activity.

ALBERT *et al.* observed that reactivation occurs also after treatment with different anti-thyroid substances, e. g. 2-thiouracil, 6-*n*-propyl-thiouracil, aminothiazole, and KSCN. As all these substances are reducing compounds, and as the recovery of the TSH-activity occured immediately after the reduction of the iodine, loosely bound with the protein precipitate, other reducing substances having no anti-thyroid properties, were investigated too. And indeed ascorbic acid and sodium thiosulphate could partly restore activity, together with reduction of the bound iodine and reversal of the insolubility of the proteins. NaOH bleaches and dissolves the precipitate, but does not restore the activity. Further, the question whether reactivation was perhaps a hyperactivation of a small quantity of TSH that had escaped the iodine action was answered by ALBERT[1] *et al.* They observed that reactivation after treatment with anti-thyroid substances is much greater than after treatment with corresponding quantities of other reducing substances; e. g. vitamin C did not reduce sufficiently to restore the normal TSH-activity. Furthermore, it was shown that the increased TSH-activity after treatment with anti-thyroid substances still remained after removal of the reducing substances by dialysis. They observed also the peculiar phenomenon that KSCN, although restoring the TSH-activity, causes no hyperactivation. In this connection they point to the selective action of this anti-thyroid substance, namely blocking of the iodide-concentration and not of the iodization.

After these experiments *in vitro* ALBERT *et al.*[2] made a number of experiments *in vivo*. Injection of a definite quantity of TSH into chickens treated with thiouracil caused a significantly higher thyroid activity than in untreated controls receiving the same quantity of TSH. This can, of course, be explained by the block of the TH-synthesis in the first-mentioned animals, followed by a higher secretion of TSH by their own anterior pituitary. But in contradiction to this an iodized TSH-preparation (inactive in untreated chickens) was shown to be active in chickens treated with thiouracil.

It is obvious that such investigations can explain many of the processes, entirely mysterious up to a few years ago, that play a part in the hormone synthesis of the thyroid-cell. Especially the rôle of TSH has been greatly elucidated, as a few years ago we knew only that TSH increased thyroid activity.

But the observations relating to the rôle of iodine are also important. In medical practice a theoretically incomprehensible contradiction existed: treatment of hypothyroidal patients with the iodine-containing compound thyroxine together with treatment of hyperthyroidal patients with Lugol-solution (I-KI). It is possible that the inactivation of TSH by iodine plays a

[1] J. WERNER, Trans. Chem. Soc. *101*, 2166 (1912).
[2] R. W. RAWSON *et al.*, Endocrinol. *39*, Abstract 42 (1946).
[3] A. ALBERT *et al.*, Endocrinol. *40*, 299 (1947).

[1] A. ALBERT *et al.*, Endocrinol. *40*, 303 (1947).
[2] A. ALBERT *et al.*, Endocrinol. *40*, 361 (1947).

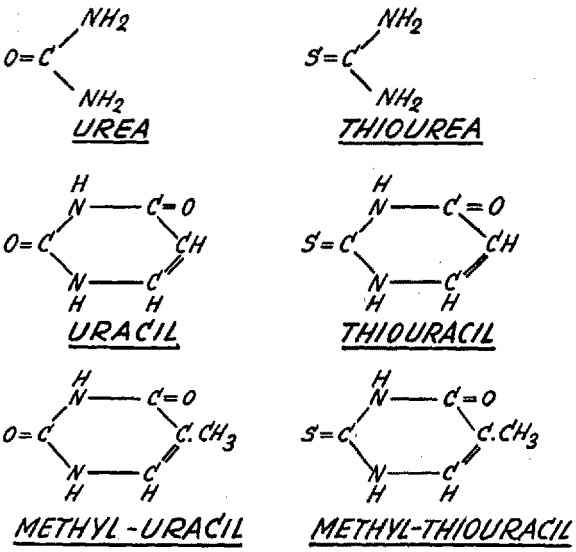
part in the last-mentioned treatment. Not much can be said definitively on this subject, but we can hope that these questions too will be solved before long.

(*d*) *Influence on the bio-synthesis of nucleic acids.*— The supposed fourth influence is connected with the nucleic acids, which play such an important part in nuclear division and protein formation within the cells. These nucleic acids contain phosphoric acid, carbohydrates, and nucleotides. Two types of nucleic acids are distinguished: pentosenucleic acid and desoxypentosenucleic acid (in the first the carbohydrate is pentose, in the second desoxypentose). Pentosenucleic acid contains nucleotides derived from guanidine, adenine, cytosine, and *uracil*, desoxypentosenucleic acid contains nucleotides derived from guanidine, adenine, cytosine, and *methyl-uracil*. It can easily be seen that many endocrinologists who had heard the names of two of the most important anti-thyroid substances: thiouracil and methyl-thiouracil, thought of a connection with these nucleic acids. The first was POUPA[1], and he developed a special theory about the influence of the anti-thyroid substances. These were supposed to have, beside their central influence on the thyroid, a perpiheral one on the body cells. He claimed that the body cells would assimilate thiouracil instead of uracil. He saw an analogy with the phenomenon that certain microbes assimilate instead of *p*-aminobenzoic acid the corresponding sulphur-compound sulphanylic acid.

The same hypothesis can be found in a publication of LAUFER and STEWART[2]. They call the supposed action: "antibiotic". They point to the fact that ethyl- and propyl-thiouracil are less toxic than thiouracil. These substances differ from thiouracil and methyl-thiouracil in having the longer ethyl- and propyl-groups and, therefore, have no antibiotic influence.

RERABEK and RERABEK[3] are of the same opinion. They determined the contents of pentose- and desoxypentosenucleic acid in thyroids of rats, treated and untreated with methyl-thiouracil. It was found that after treatment the contents of both substances had only half the normal values. These facts speak for an antibiotic action. Against this conclusion we must raise an objection, namely that the authors have taken no account of the fact that the increase in weight of a thyroid gland after treatment with anti-thyroid drugs is caused not only by increase of the number of follicles, but often for the greater part by increase in vascularity and thus in blood-content.

Yet there are a number of investigations pointing to a real connection between nucleic acids and anti-thyroid substances. BEVELANDER[4] and SOBELS[5] observed an influence on nuclear division in eggs of *Arbacia punctulata* and *Limnaea stagnalis* respectively treated with thiourea-solutions.




POUPA and JELINEK[1] studied the problem in another manner. They observed that yeast-ribonucleic acid accelerated the growth of tadpoles, without affecting metamorphosis. To make it possible to study the direct "antibiotic" effect of thiouracil on the nucleic acid synthesis these investigators[2] let yeast grow on a synthetic culture medium, to which thiouracil was added. The nucleic acid extracted from this yeast showed aberrant properties, seen notably in the fact that metamorphosis of tadpoles was actually retarded. Perhaps new investigations will throw more light upon these questions as well.

### 4. *Discussion*

On the basis of the results of the investigations mentioned above we can conclude with certainty on what parts of the hormone synthesis in the thyroid cell the anti-thyroid substances act: namely the thiocyanates on the iodide concentration, and the thiourea-derivatives on the iodization and also on the activity of TSH. The last fact is often considered to be of secondary importance. For forming a good idea of the morphological alterations in the thyroid gland during treatment with anti-thyroid substances (viz. the enormous increase of histological activity—increase of cell height, colloid secretion, mitosis, blood content, and so on—), it is of the first importance to know that TSH is hyperactivated, and that a possible inactivation by iodine in the thyroid cells will immediately be repaired by the anti-thyroid substances.

Not only these facts, but many others also (e. g. the deficit of TSH in the urine of hyperthyroid persons and the

[1] O. POUPA, Sbornik Lekarsky *48*, 219 (1946).
[2] L. LAUFER and E. D. STEWART, Science *105*, 327 (1947).
[3] J. RERABEK and E. RERABEK, Acta physiol. Scand. *14*, 276 (1947).
[4] G. BEVELANDER, Proc. Soc. exper. Biol. Med. *61*, 268 (1946).
[5] F. H. SOBELS, Proc. Kon. Akad. Wetensch. Amsterdam *51*, 900 (1948).

[1] O. POUPA and O. JELINEK, Biol. Listy *28*, 40 (1947).
[2] O. POUPA and O. JELINEK, Lekaru ceskych. *87*, 744 (1948).

increased content of TSH in urine of hypo- or athyroid persons) suggest that TSH is consumed by the thyroid cells during hormone synthesis. It is even thinkable that TSH is the protein factor besides iodine indispensable for the formation of thyreoglobulin (cf. LEVER[1]). If after comprehensive investigations this hypothesis should be shown to be right, then the central processes of the hormone synthesis and its regulation will be not only explained, but even reduced to one process. As yet this cannot be concluded with certainty. The fact that it is possible to lend probability to such a working hypothesis with different arguments, shows very clearly that during the last years thyroid endocrinology has made considerable progress.

The last mentioned influence of the anti-thyroid substances, namely their supposed "anti-biotic" effect, has unlocked a new and specifically cytological field of exploration in thyroid research, as with this one may attempt to explain not only the secondary symptoms of treatment with these drugs (e. g. agranulocytosis), but also some morphological changes in the thyroid gland (RERABEK and RERABEK[2]). Against this idea we must raise several objections: in the first place, although there exists a direct chemical relation between uracil and thiouracil, and between methyl-uracil and methyl-thiouracil, this can hardly be extended to substances as aminothiazol, the thiocyanates, or the sulfonamides.

But we must raise an objection also against the hypothesis that the changes in cytological structure have to be ascribed to the anti-thyroid substances.

Before discussing this important question we must describe first some functions of the nucleic acids.

It is evident that these substances are indispensable for every form of protein synthesis in living cells, this process being chiefly located in the nucleus, as one of its most important functions. The desoxypentosenucleic acid plays a considerable part at chromosome reproduction during mitosis: at the beginning of the prophase much of this substance becomes attached to specific loci of the polypeptide-chains forming the chromosome threads, and probably causes their shortening by spiralization, which causes the chromosomes to become visible after staining: maximum attachment coincides with maximum spiralization of the chromosomes at the end of the metaphase. The detachment of nucleic acids begins at the late anaphase and increases through the telophase, coinciding with despiralization of the chromosomes. In accordance with these facts, CASPERSSON[3] observed an increase in nucleic acid concentration during the earliest phases of the mitosis cycle and a decrease during the telophase. The increased chromophily of the nucleus during the earliest phases of mitosis is caused by this increase of nucleic acid.

The quantity of desoxypentosenucleic acid varies along the chromosome. It is always retained by specific parts; these parts form the heterochromatin, which is always visible in microscopic preparations. The function of heterochromatin is described by CASPERSSON[1] as follows: it secretes substances of a protein nature, showing pentosenucleic reactions. These substances accumulate and form the main part of the nucleolus. From the nucleolus the pentosenucleic acid diffuses towards the nuclear membrane, on the outside of which an intensive protein synthesis, located around pentosenucleic acid-containing particles, takes place, through which the content of cytoplasmatic proteins increases.

During mitosis this heterochromatin again has an important function. After chromosome duplication, when both daughter chromosomes are lying side by side, protein synthesis at the heterochromatin probably causes dispersion of the chromosomes and thus the beginning of the anaphase (DARLINGTON[2]).

After this short review it is obvious that it is possible to reach several more physiological conclusions on the basis of morphological data, for example:

(*a*) On the basis of the size of the nucleoli one can say something about the quantity of synthesized pentosenucleic acid and about the protein synthesis in cytoplasm.

(*b*) As both types of nucleic acids have about the same constituents, it is understandable that during mitosis, when much desoxypentosenucleic acid is synthesized, no pentosenucleic acid can be made: thus the nucleolus disappears during mitosis; conversely, if there are many mitoses one can conclude that few plasma proteins are formed.

(*c*) The relative frequencies of mitotic stages give a measure of their relative duration and of the content of desoxypentosenucleic acid. If the content of desoxypentosenucleic acid is high, e. g. in the nuclei, the duration of the prophase (during which the attachment of desoxypentosenucleic acid to the unspiralized interphase-chromosomes takes place) can be short. An excess of desoxypentosenucleic acid can cause the phenomenon of a nucleus failing to pass through the metaphase, since the chromosomes are overcondensed and clump together (e. g. the action of colchicine, KOLLER[3]).

(*d*) "The well-known staining of fixed nuclei with basic dyestuffs indicates the presence of liberated acid groups" (FREY-WYSSLING[4]).

[1] J. LEVER, *Onderzoekingen betreffende de schildklierstructur*, Thesis (Utrecht, 1950).

[2] J. RERABEK and E. RERABEK, Acta physiol. Scand. *14*, 276 (1947).

[3] T. CASPERSSON, Skand. Arch. Physiol. *73*, suppl. 8 (1936).

[1] T. CASPERSSON, Symp. Soc. exper. Biol. I, 127 (1947).

[2] F. S. DARLINGTON, Endeavour *8*, 51 (1949).

[3] P. C. KOLLER, Symp. Soc. exper. Biol. I, 270 (1947).

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(*e*) If the size of the nuclei in a tissue is smaller than normal, this often indicates a decreased protein synthesis.

RERABEK and RERABEK[1] gave cytological data about thyroid cells in connexion with the theory mentioned above that anti-thyroid substances would have an "antibiotic" influence on nucleic acid synthesis. They observed that the nuclei in thyroid cells of rats, after treatment with methyl-thiouracil, were larger, that the number of mitoses was increased, and that the number of cells in metaphase was higher than that of cells in other phases of mitosis, from which they concluded that metaphase was blocked.

A similar investigation (LEVER), also with application of other anti-thyroid substances, showed after 10–14 days of treatment of chickens that

(*a*) Low concentrations of these anti-thyroid substances (e.g. thiourea, thiouracil, methyl-thiouracil) cause an increase in nuclear size, but high concentratious cause a decrease again.

(*b*) The number of mitoses increases in direct relation to the concentrations of the drugs used.

(*c*) The number and size of the nucleoli decrease, from which it can be concluded that there is a decrease in synthesis of cytoplasmic proteins.

(*d*) The number of cells in metaphase in normal and in treated animals is higher than that of cells in prophase or ana-telophase; thus the duration of metaphase is longer than either prophase or ana-telophase in normal *and* in treated animals; moreover the desoxypentosenucleic acid content in the nuclei is high (short prophase).

On the ground of these facts one would like to conclude that anti-thyroid substances do indeed cause a number of characteristic alterations in the thyroid cells.

*But with repeated injections of TSH exactly the same results were obtained as those listed above under a–d* (increase in nuclear size, decrease in number and size of the nucleoli, increase in the number of mitoses).

From this we have to conclude that the alterations in the structure of thyroid cells found in animals treated with anti-thyroid substances must be caused by the increased TSH-delivery of the hypophysis.

So the hypothesis that these substances have an "anti-biotic" action on nucleic acid synthesis (dependent on their relation with uracil and methyluracil, parts of the molecules of pentosenucleic acid and desoxypentosenucleic acid respectively) is not sustained by these morphological arguments. It is not necessary to reject this theory completely, but we must assume an attitude of scepticism towards it.

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*Zusammenfassung*

Während des Krieges und nach dem Kriege wurden mittels zweier experimenteller Hilfsmittel (das radioaktive Jod und die antithyreoiden Stoffe) bei der Untersuchung der Schilddrüse sehr bedeutende Fortschritte erzielt.

Es ist jetzt möglich, bei der Hormonbildung folgende Prozesse zu unterscheiden: die Jodidkonzentrierung, die Thyreoglobulinbildung, die Thyreoglobulinspeicherung, die Thyreoalbuminbildung und die Thyreoalbuminabgabe.

Die antithyreoiden Stoffe beeinflussen mehrere Teile der Hormonbildung. Nacheinander werden besprochen: die Blockierung der Jodidkonzentrierung, die Hemmung des Jodierungsprozesses, die unmittelbare Wirkung auf das TSH (thyreoidstimulierendes Hormon) und die Wirkung auf die Biosynthese der Nukleinsäuren.

Die zuletzt erwähnte Auffassung wird ausführlich besprochen, weil sie neuerdings mehr und mehr beachtet wird.

Eine Untersuchung des Autors zeigte, daß nach Verabreichung verschiedener antithyreoider Stoffe in der Tat eine Anzahl charakteristischer Veränderungen in den Thyreoidzellen verursacht wurden (Kernvergrößerung, Verringerung und Verkleinerung der Nukleoli, zahlenmäßige Zunahme der Mitosen). Es stellte sich aber heraus, daß wiederholte Injektion von TSH genau dieselben Erscheinungen verursacht. Hieraus ergibt sich wieder, daß die Veränderungen, die unter dem Einfluß antithyreoider Stoffe in der Kernstruktur auftreten, durch erhöhte Absonderung von TSH durch die Hypophyse verursacht werden. Morphologisch betrachtet erhält die Theorie der «antibiotischen» Wirkung der antithyreoiden Stoffe bis jetzt noch keine Unterstützung.

[1] J. RERABEK and E. RERABEK, Acta physiol. Scand. *14*, 276 (1947).