

CHEMICAL
DEVELOPMENTS
in
THYROIDOLOGY

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AMERICAN LECTURES IN ENDOCRINOLOGY

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INTRODUCTION

IT HAS been said that in this century the mathematicians are becoming philosophers; the physicists mathematicians, the chemists physicists, and the physiologists chemists. In the field of endocrinological investigation, formerly the province of the old-time mammalian physiologist, this drifting of the sciences has also made itself apparent. The past decade has seen great strides in the study of thyroid physiology and thyroid disease. Most of this progress, however, has been made with tools and methods that are primarily chemical in character. Indeed, the investigative approach has even drifted into the field of nuclear physics. It is the purpose of this essay to describe some of these interesting developments.

To this end, attention will be focused upon four main types of study, which can be catalogued as follows:

- (1) Short-cut synthesis of the thyroid hormone by methods which imitate nature.
- (2) The development of blocking agents which impede the natural synthesis.
- (3) Studies of the peripherally circulating hormone.
- (4) The application of radio-iodine to problems in physiology and therapy.

None of these trends is yet completed and it may be some time before the whole story can be written of each. Nevertheless, already so much material has accumulated that the inner working of the thyroid gland is becoming much more comprehensible. Moreover, these trends interdigitate so well

that a new biochemical era in thyroidology seems to have arrived.

To be sure, the dawn of this era had been foreshadowed even before the Global War broke. For instance, nearly a decade ago the author summarized the existing knowledge of thyroid function (93) and emphasized the "Endocrine Function of Iodine" (94). The humble beginnings of that time, however, have now blossomed and borne fruit far beyond his highest expectations. For example, the only pure goitrogenic agents considered in that treatise were thiocyanate and certain cyanides. Furthermore, the use of radio-iodine in human therapeutics was mentioned only as a dream. It is the purpose of this essay to describe these recent advances and to hazard some evaluation of them.

SYNTHESIS OF THE THYROID HORMONE

EVER SINCE Baumann (13) discovered in 1895 that the thyroid contained iodine and, as he correctly surmised, its essential principle likewise, attempts have been made to produce thyroid activity by incorporating iodine into organic materials. Baumann himself (14) attempted to increase the activity of the thyroid globulin by iodinating it further, but without success. Later other investigators tried the same experimental approach. Notable among these was Oswald, who about 1911 published a long series of investigations on iodinated proteins (82) (83) (84). He noted that there were several steps in the iodination of the protein. He noted, further, that some of the iodine was bound to nitrogen, probably as iodohistidine. He was unable, however, to demonstrate the production of endocrinological activity in these proteins. For this reason interest in the iodinated proteins ultimately lapsed. Nevertheless, the general hypothesis lay in the back of biochemists' minds, namely, that by introducing iodine into protein a remarkable endocrine activity might be produced.

The essential question was how this remarkable property could arise; and, in particular, what was the chemical mechanism by which the living gland effected such a synthesis. From time to time, this problem recurred and several investigators thought they had produced weak endocrine potency through the iodination of impotent material. The most outstanding of these preliminary investigations was that of Abelin (2) (3), whose iodinated proteins hastened the metamorphosis of tadpoles. When Lerman and Salter (66) showed that the iodination of serum protein could yield in

a few minutes a preparation which relieved classical human myxedema in striking fashion, there no longer remained any doubt that iodinated protein could indeed contain effective hormonal activity. Moreover, quite recently, Dvoskin (32) has relieved experimental myxedema by intramuscular injection of elementary iodine in solution. In 1936 Ludwig and von Mutzenbecher (69) iodinated casein and from the hydrolysate thereof isolated crystalline racemic thyroxine, as well as crystalline diiodotyrosine and (probably) crystalline monoiodotyrosine. The isolation of the crystalline thyroxine was confirmed by Harington and Pitt Rivers (46) and by several other investigators in America. Consequently there no longer was any doubt that the genesis of thyroxine could occur during the iodination of protein. The problem then resolved itself in two separate developments. The first of these was to improve the yields of the original process. The second was to unravel the chemical mechanism by which this rapid synthesis of thyroid hormone occurred.

In the original procedure the yield of thyroxine obtained by Ludwig and von Mutzenbecher was about one-tenth of one per cent in terms of the total iodine involved. Subsequently Block (16) obtained a sample of crystalline thyroxine from pure iodotyrosine which had been incubated in an alkaline medium. This yield, however, was only about one-tenth of one percent. Such small yields were disappointing, even though the qualitative mechanism of production had been demonstrated adequately. Thereupon there ensued a series of experiments designed to increase the efficiency of this synthetic process.

The outstanding work along these lines was conducted by Turner and Reineke (109), (110), (88), and was concerned mostly with the iodination of casein. A systematic study of the reaction was made. Such observations showed that there are several factors which influence appreciably the

yield of hormone. Among these are the acidity of the medium, the temperature of incubation, the degree of aeration of the mixture, the rapidity of stirring, and the extent of the iodination. In addition, some evidence has been adduced which suggests that the reaction is catalyzed by compounds of manganese. Before discussing in detail the influence of these variables, it should be stated at once that these studies have confirmed amply the original hypothesis of Harington and Barger (44). Those investigators suggested that thyroxine should arise in nature by the conjugation of two molecules of diiodotyrosine. Now that the process has been demonstrated *in vitro*, the time is ripe to extend the basic procedure along biosynthetic lines.

The conditions under which this synthesis occurs in the laboratory involve a mild oxidation and the elimination of one of the alanine side-chains of the two diiodotyrosine molecules. This fact is of considerable biological interest because it suggests the mechanism by which the gland itself operates in the elaboration of this specific hormone.

As to the optimal conditions under which iodinated proteins produce maximal yield, the following are those indicated by Reineke's studies (108) of the process. The pH should lie between 6.8 and 8.0. The temperature during iodination should be kept between 30 and 45° C. The medium should be buffered with sodium bicarbonate while the iodine is added. Approximately four to five atoms of iodine are required per mol. of tyrosine. A subsequent incubation with stirring for eighteen to twenty hours is then carried on at a temperature between 50 and 100° C. It seems likely, also, that the process can be expedited by some catalyst: possibly copper, tin or zinc. When these conditions are followed as closely as possible, the production of crude thyroxine is approximately three percent, if manganese tetroxide is used as a catalyst.

This result has been confirmed both by biological and chemical assays. However, the actual yield of crystalline thyroxine on isolation is considerably lower because of inherent losses in the process of isolation. In fact, yields of d, l-thyroxine higher than 0.5 percent have not yet been obtained. Moreover, even to obtain such yields it has been necessary to use the classical isolation procedure which involves alkaline hydrolysis with barium hydroxide. This alkaline hydrolysis racemizes the natural optically active thyroxine: so that the final product is a racemic mixture. Because biological assays indicate that pure l-thyroxine has twice the potency of the d, l-mixture, it would be desirable to obtain thyroxine solely in the form of the levorotatory isomer. This preparation has in fact been performed by heating the iodinated protein with sulfuric acid. The resulting hydrolysis, however, is not so efficient and the recoveries of pure l-thyroxine by this process are considerably lower than those obtained from the alkaline baryta. Indeed, even allowing for the higher activity of the levorotatory isomer, the total activity so obtained is less than that of the d, l-combination. So much for the isolation of the hormone from iodinated protein.

Of considerable theoretical interest, moreover, is the fact that crystalline thyroxine can be synthesized from pure diiodotyrosine. This finding simplifies considerably the chemist's problem, namely, by what mechanism diiodotyrosine turns into thyroxine. The importance of this question for the biochemist lies in the fact that it gives a clue to the internal physiology of the thyroid itself.

Because the synthesis of active hormone from casein can be pursued on a large scale, agriculturists are interested in the possibility that it might be used in animal husbandry. Consequently, a number of experiments are now in progress to determine how profitably such material may be employed

by dairies and farmers. In England (15) (85) a series of experiments has been conducted with various starting materials, including a powder obtained from "ground nuts." In the United States most of the work has been limited to iodinated casein. Preliminary results indicate that apparently some increased production of dairy products can be effected with this material. For example, in laying hens the synthetic material appears to counteract somewhat the decline of egg production which occurs during hot weather. Similarly young hogs have grown a little more rapidly and chickens have shown somewhat faster rates of body growth and marked increases in feather growth. In lactating animals, e.g., cows and goats, a consistent increase has been shown in the production of milk, and particularly of the milk fat. It remains to be learned, however, whether the cost of such treatment is justified by the increased production. This cost, obviously, must be calculated in terms of both the medication and the increased metabolic turnover which ensues. The answer is by no means clear as yet.

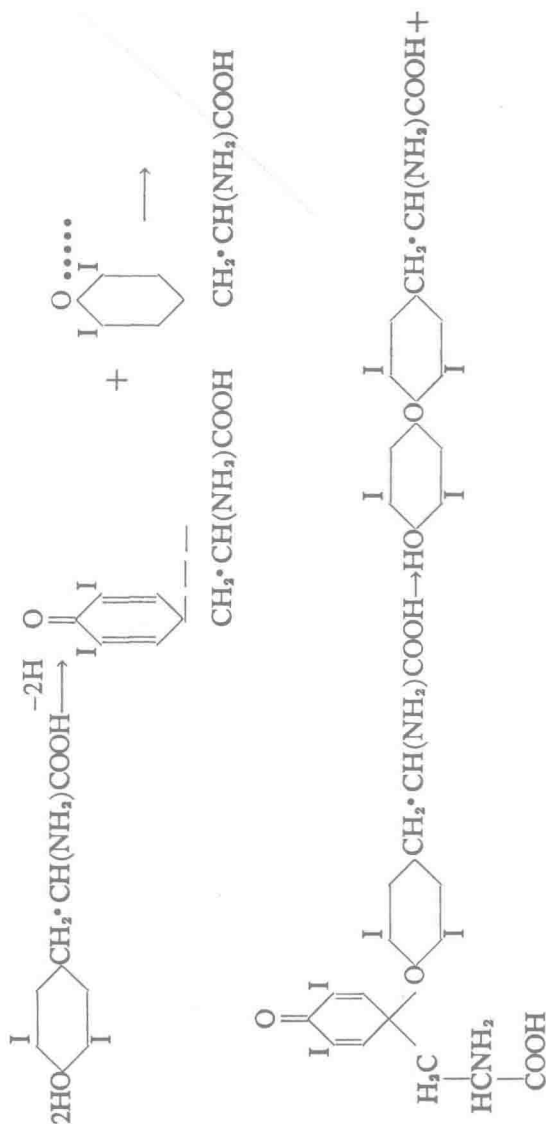
Apart from these practical considerations, however, the pure scientist and the clinical investigator are more interested in the implications which the reactions thus demonstrated may have for physiology and pathological physiology. This problem has been considered by a number of investigators using various chemical approaches. One of the earliest and most fruitful of these was the study of Johnson and Tewkesbury (57). They started with the knowledge that certain essential features were needed for thyroid activity. The chief of these, as outlined by Harington (42), was the presence of the organic configuration known as "thyronine." Its structure is illustrated in the following formula.



Many studies have shown that this general framework in the molecule is relatively essential for potency. For example, through the synthesis of "iso-thyroxine," i.e., β , β -di (3, 5-diiodo-4-hydroxyphenyl) α -amino-propionic acid, it was shown that the mere presence of all the essential atoms and chemical groups was not enough. As indicated in the formula just given, this compound contains two phenyl groups directly linked to carbon instead of in the classical diphenyl-ether combination. The test material, synthesized by Harington and McCartney (45), proved to be inert physiologically. Therefore, a considerable part of the present problem consists in determining how two molecules of diiodotyrosine can combine to form thyroxine. As a matter of fact, it is not necessary to have four iodine atoms in thyroxine in order to achieve activity. It was shown, for example, by Anderson, Harington and Lyon (5) and by Lerman and Salter (67) that diiodothyronine is fully effective if given in adequate doses. Nevertheless, the activity of this compound is only one-twenty-fifth that of thyroxine itself. How, then, does the diphenyl-ether structure arise from two molecules of diiodotyrosine?

As a tentative answer to this question Johnson and Tewkesbury (57) proposed an oxidative mechanism. They made certain assumptions based upon the work of Pummerer (86), who studied the oxidation of o- and p-substituted phenols in alkaline solution. Pummerer had adduced evidence that a quinol-ether intermediate might be formed under such circumstances. Accordingly, Johnson and Tewkesbury oxidized 3,5-diiodotyrosine with hypiodous acid (HOI). Under these circumstances in addition to the quinol-ether, ammonia and puruvic acid should be formed, as indicated in Fig. 1.

These two substances were actually identified in their reaction mixture.



$$\text{CH}_2 = \text{C}(\text{NH}_2)\text{COOH} \xrightarrow{\text{H}_2\text{O}} \text{CH}_3\text{C}(\text{:NH})\text{COOH} \rightarrow \text{CH}_3\text{CO}\cdot\text{COOH} + \text{NH}_3$$

Fig. 1. Hypothetical conversion of diiodotyrosine to thyroxine.
 After Johnson and Tewkesbury, *Proc. Natl. Acad. Sci. U.S.*, 28:74, 1942.