The Study of Iodine Metabolism IN VIVO Utilizing I-131

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방사선 동위원소 I—131을 이용한 요드의 IN VIVO 대사 연구

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SUMMARY

In order to study the mechanism of biosynthesis of thyroid hormones, radioactive iodine was injected into the rats and thyroid glands were removed. Iodine compounds hydrolyzed by pancreatin viokase were separated by paper chromatography and analyzed by radioautography. Radioautograms showed that the uptake of iodine starts immediately and forms diiodotyrosine through monoiodotyrosine.

Evidence supported the possibility that diiodotyrosine is a precursor of thyrosine and triiodothyronine is a degradation product of thyroxine.

The rat administered propylthiouracil showed inorganic iodine concentration activity, while the binding activity was prevented.

INTRODUCTION

The isolation of thyroxine by Kendal(1), its identification as tetraiodothyronine(T_4) by Harington(2) and its synthesis gave to the biochemistry of thyroid hormone a fundamental impulse. The extensive use of one of the radioistopes of iodine, I-131, in biochemistry, physiology, and medicine led to important progress in nearly every aspect of thyroid biology.

However, many of the problems of the basic mechanism of biosynthesis are not solved completely and in detail, even though the mechanism of the formation of thyroid hormone was one of the main goals of biochemical research on the gland, as soon as a first active product had been extracted from it.

The biogenesis of thyroid hormone in the thyroid gland can be suggested in two ways(3). The first one is that of Harington and Barger(2), and Morton et. al. (4), who considered 1 or 3, 5-diiodotyrosine (DIT) to be a precursor of 1-thyroxine (tetraiodothyronine, T_4). The other is biosynthesis of 1 or 3,5, 3-triiodothyronine (T_3) through DIT which converts to T_4. However, it was very doubtful that this proposed T_3 is present in small quantities.

In the mechanism of iodide accumulation, so

called, "iodide pump" or "iodide trap" by thyroid tissue, Taurog *et. al.* showed that the concentrating capacity is dependent upon the gland's ability to convert inorganic iodine to DIT and T_4, by using inorganic radioactive iodine, and that this mechanism is independent of the iodide binding mechanism by administering the reducing agent propylthiouracil (PTU). Also Franklin *et. al.* (6) observed that in the presence of p-aminobenzoic acid, thiourea, and sulfanilamide, thyroid slices failed to convert inorganic iodide to DIT and T_4, but retained their capacity for concentrating iodine as in PTU. On the other hand, thiocyanate, perchlorate, and periodate act to prevent the concentration of iodide.

In this condition, as shown by Vanderlaan and Bissel(7) the iodine take-up does not remain in the gland for long, the maximum concentration being reached within the first half-hour after the iodine injection.

More recently many investigators found the iodination of tyrosine in the thyroid gland. By analogy with iodination reactions carried out in the laboratory, it is generally believed that iodide ion must first be oxidized to a higher oxidation state before it can iodinate tyrosine. Of the known biological oxidizing agents, only hydrogen peroxide and oxygen are involved in oxidation-reduction potential that hydrogen peroxide has longbeen suspected as the agent responsible for oxidizing iodide in the thyroid.

Kirkwood *et. al.*(8). proposed "tyrosine iodinase" as the active enzyme in this process and also Alexander (9) and Serif and Kirkwood (10) reviewed the occurance of a thyroid peroxidase. The existence of thyroid peroxidase has been confirmed further by other workers(11, 12, 13). Taurog *et. al.* (14) and Shaw and Hager (15) investigated that chloroperoxidase, also is very active in this process.

In this experiment, related observations have been made on rat thyroids *in vivo* studies by utilizing radioactive iodine, I-131.

EXPERIMENTAL PROCEDURE

Materials; Chemicals were obtained from the following sources: 1-butanol, dioxane, tris-(hydroxymethyl)-aminomethane and p-anisidine from Fisher Scientific Co. propylthiourea, KI, DIT, MIT, T_3, and T_4 from Sigma Chem. Co; pancreatin(viokase) from Vioken Corp.; and radioactive iodine, I-131 from Oak Ridge National Institute.

Methods; Rats that weighed about 200 grams were lightly anesthetized with ether, injected intraperitoneally with 100μCi of I-131 in 0.5ml. of 0.9% Nacl and killed with ether according to time schedule. 15 minutes, 2 hours, and 24 hours after injection. At the same time, we began feeding a rat on a diet containing 0.05% propylthiouracil and next day it was injected with 100 μCi of I-131 as before and killed 2 hours after injection. The thyroids are removed, placed in a small vial, frozen by touching to dry ice and stored in the refrigerator at 15°C.

Homogenization and digestion of thyroid glands; As previously digested with pancreatin reagent by Tong and Chaikoff (16), the fresh tissues were homogenized with 0.3ml. of ice cold tris buffer (pH 8.3). Insoluble material was removed by centrifugation at 5,000 x G for 5 minutes at 2°C. Without disturbing the precipitate the supernatant was transferred and then digested with pancreatin viokase at 37°C for 24 hours by adding a small drop of toluene The digested sample was stored frozen in the refrigerator.

Chromatographic analysis and radioautography; Aliquots of thyroid gland digests and standard iodocompounds are spotted on 9" x 22" strips of Whatman No. 1 paper and developed by the descending technique. Chromatography is carried out for 10-15 hours using a 1-butanol: dioxane: 2N-NH₄OH(4 : 1 : 2, V/V) solvent. The air dried chromatograms are exposed to 14" x 17" sheets of Kodak Royal Blue X-ray film for 24-48 hours by the procedure of Taurog and Howells. [14] After radioautography the chemical detection of iodine compounds is based on the observation of Sandel and Kolthoff (17) that iodine markedly

catalyzes the oxidation of arsenious acid by ceric sulfate in sulfuric acid solution according to the modification of Bowden (18) to improve the stability after mixing.

RESULT AND DISCUSSION

In order to observe biosynthesis of thyroid hormones radioactive iodine materials were injected into rats and the thyroid glands were removed according to the appropriate time schedule. After the treatment of pancreatin reagent the paper chromatograms obtained were applied to radioautography. Resulting radioautogram and paper chromatograms are seen in Figure 1-3.

The radioautogram shown in Figure 1 elucidated the radioactive iodine uptake clearly according to the time of tracer iodide injection. After injection

Figure 2. Paperchromatogram of each sample prepared from pancreatin viokase digested thyroid gland. Descending chromatography was carried out for 15 hours using 1-butanol· dioxane: 2N-NH₄OH (4 : 1 : 2) solvent. Each spot is labelled as follows; 1. unknown; 2. diiodotyrosine (DIT); 3. monoiodotyrosine (MIT); 4. inorganic iodide (KI); 5. thyroxine (T₄); 6. triiodothyronine (T₃).

of a tracer dose of iodide, the uptake of iodide and formation of MIT and then DIT in turn, starts almost immediately and reaches a maximum within a few hours as shown in the 15 minutes and 2 hours fractions. These results have been observed by Taurog et. al. (8, 27). In the fractions, 15 minutes, 2 hours, and 24 hours time schedule, the radioautographic analysis showed that the formation of DIT is somewhat slower than MIT but faster than T₄ by spot thickness, so that inorganic iodide would incoporate with tyrosine first in this condition to form MIT, and MIT is the precursor of DIT, and then T₄ was formed from DIT in turn. T₃ occured much later (24 hours), at the time when other iodine compounds are decreasing. Therefore, T₃ is not seen to be a precursor of T₄ and is a degradation product of T₄. This results supported the evid-
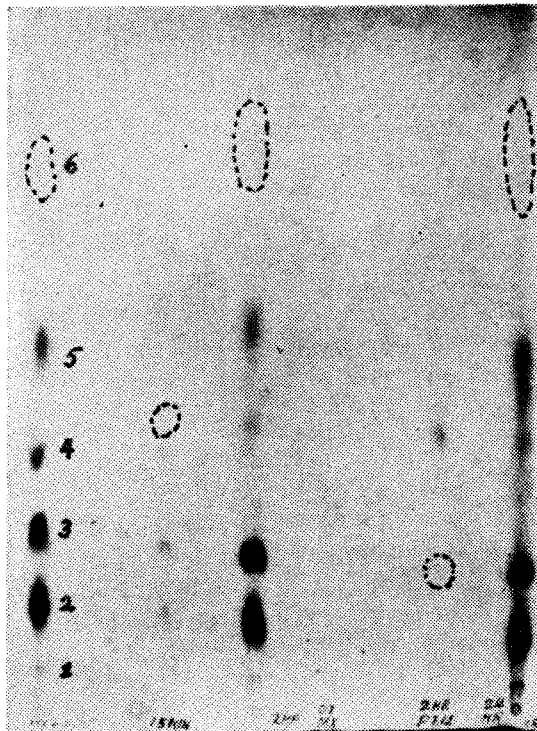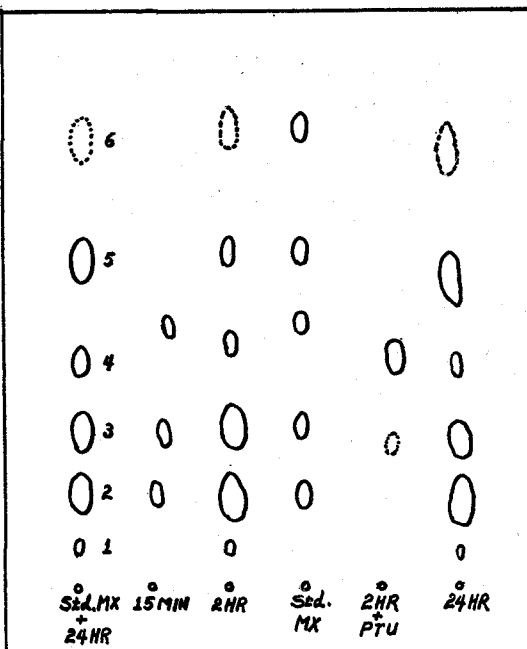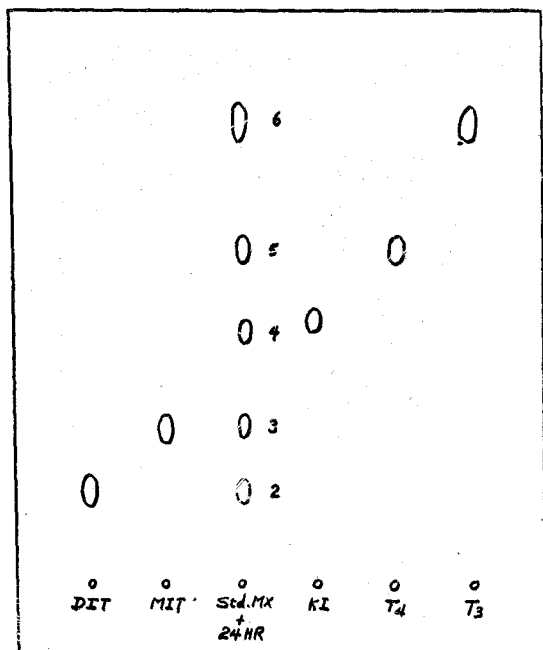
Figure 1. Radioautogram of each sample prepared from pancreatin viokase digested thyroid gland. Descending chromatography was carried out for 15 hours using 1-butanol: dioxane; 2N-NH₄OH (4 : 1 : 2) solvent. Each spot is labelled as follows; 1. unknown; 2. diiodotyrosine (DIT); 3. monoiodotyrosine (MIT); 4. inorganic iodide (KI); 5. thyroxine (T₄); 6. triiodothyronine (T₃).

Figure. 3. Paperchromatogram of standard compounds. Descending chromatography was carried out for 15 hours using 1-butanol; dioxane: 2N-NH₄OH (4 : 1 : 2) solvent. Each spot is labelled as follows: 2. diiodotyrosine (DIT); 3. monoiodotyrosine (MIT); 4. inorganic iodide (KI); 5. thyroxine (T_4); 6. triiodothyronine (T_3).

ence of Harington et. al. (2) that thyroxine is formed in the thyroid gland from tyrosine with diiodotyrosine as an intermediate.

The rat which was administered PTU showed a heavy inorganic iodide spot and only a trace of MIT while normal rats showed most of DIT and MIT at 2 hours. By this experiment it is seen clearly that PTU acts in the prevention of binding activity of inorganic iodine into organic iodine.

This, however, indicated that PTU did not interfere with the iodide concentrating mechanism, so that the radioautogram showed the KI spot heavily.

In the paper chromatogram, the KI compound showed that it moved faster in the pure solution than in the mixture of samples. This fact might be partly due to some interference of sample solution and partly due to the differrence of solvent frontline in the two spots. In any case, KI showed always a slightly yellow color before applying chemical reagent on the chromatogram. We can observe one weak spot on the bottom of the paper chromatogram, near the original sample spot, which was found in the refernence (16). This is probably a peptide hydrolyzed by pancreatic viokase action.

In order to observe in detail the mechanism of biosynthesis of thyroid hormones, further extensive work will be needed such as detection of thyroid peroxidase and guantitative experiments with radioactive iodine.

요 약

Thyroid hormone의 생합성반응기작을 알아보기 위하여 방사선 I-131을 쥐에 주사한 후 thyroid gland을 분리하여 분석하였다. Pancreatin viokase 를 처리하여 분리한 요드화합물을 여지 크로마토그라피로 분리 동정하고 이를 radioautography로 분석한 결과 I-131은 주사후 바로 쥐의 체내에서 흡수되어 monoiodotyrosine이 되고 이것은 diiodotyrosine으로 전환됨을 관찰하였다.

실험결과는 diiodotyrosine은 thyroxine 생합성에 있어서 중간생성물이나 반면 triiodothyronine은 오히려 분해산물임을 보여 주었다. 또한 환원제인 propylthiouracil을 투여한 쥐에서 iodine의 체내집적현상(Iodine Pump)은 현저히 증가하였으나 유기요도화합물을 전환되는 것은 저하되었다.

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