Effect of (-)-5-Vinyl-2-oxazolidinethione on the Thyroid Hormone Biosynthesis in Chicks

Tohoku Journal of Agricultural Reseach
Vol. 22, No. 3, October 1971
Printed in Japan

Effect of (-)-5-Vinyl-2-oxazolidinethione on the Thyroid Hormone Biosynthesis in Chicks

Yukio AKIBA and Tatsuro MATSUMOTO

Department of Animal Science, Faculty of Agriculture, Tohoku University, Sendai, Japan

(Received, September 14, 1971)

Summary

Influence of addition of 0.05 per cent goitrin in feed on thyroid hormone biosynthesis was investigated by using ^{131}I and compared with that of chicks fed 0.03 per cent and 0.05 per cent PTU. In PTU-fed chicks, thyroid hormone biosynthesis is markedly inhibited through inhibition in all these steps as follows; oxidation of iodide, iodination of tyrosine, and iodothyronine synthessis from iodotyrosines. In goitrin-fed chicks, the oxidation of iodide and monoiodination of tyrosine are not depressed but the thyroid hormone biosynthesis is also inhibited. In addition, the monoiodination of tyrosine would likely be increased by goitrin administration. The most disinct difference between effects of goitrin and PTU is that the amount of iodothyronines synthesized in the gland is increased in goitrin-fed chicks and is markedly depressed in PTU-fed chicks while the plasma level of throid hormone is depressed in both groups. On the basis of results, it is suggested that the hydrolysis of thyroglobulin and secretion of thyroid hormone from the gland may be decreased or at least not enhanced by administration of goitrin in chicks.

In our previous reports (1, 2), the iodine metabolism was markedly changed and the thyroid hormone secreted in the plasma was also depressed by the feeding of (-)-5-vinyl-2-oxazolidinethione (goitrin), a goitrogen in rapesseed, for 14 days in chicks. The most characteristic features in iodine metabolism in chicks fed 0.05 per cent goitrin are the increased thyroidal ^{131}I uptake and the increased thyroidal organic ^{131}I in spite of a depressed plasma thyroid hormone level. Therefore, it was mentioned that the effects of goitrin on iodine metabolism are different in mode of action from that of propylthiouracil (PTU).

The purpose of the present investigation is to clarlify the effect of goitrin on the thyroid hormone biosynthesis in the gland in the early phase after ^{131}I administration, by determing the changes of distribution of radioactive iodinated substances in the thyroid lobes. In addition, the effect of goitrin on thyroid hormone biosynthesis were compared with that of PTU.

Materials and Methods

One-hundred and twenty day-old male Single Comb White Leghorn chicks were fed a diet (0.67 μg iodide/g of diet) containing 0.05 per cent goitrin, 0.05 per cent and 0.03 per cent PTU, respectively. A dosage of 0.03 per cent PTU seems to have same potency with 0.05 per cent goitrin in hypertrophy of thyroid gland. On the 14-th day, all chicks were injected subcutaneously in the thigh with carrier free $Na^{131}I$ (10 μCi per 100 g body weight). At 0.5, 1, 2, 4, 8, and 24 hours after dosage, five chicks in each group were killed by heart puncture and the thyroid glands and plasma were analyzed. Determination of plasma per cent of protein bound iodine-^{131}I (per cent $PB^{131}I$) was performed as previously reported by Matsumoto et al. (2). To determine the distribution of radioactive iodinated substances in thyroid lobes, the thyroid glands were homogenized immediately after killing in Tris buffer, pH 8.4 (about 1 ml per 100 mg of thyroid gland) containing 0.01 M methimazole to block deiodination and digested with pancreation for 20 hours at 37°C. The aliquots of the hydrolysate was subjected to ascending paper chromatography for 16 hours in an n-butanol-absolute ethanol-0.3N NH_4OH (5:1:2) system. The chromatograms were radioautographed for 48 hours. The radioactive portions of the paper were cut into pieces and a measurement of the radioactivity was performed in a well-type scintillation detector.

The goitrin used in this experiment was prepared from rapeseed (*Brassica napus*) by the method of Astwood et al. (3).

Results

Goitrogen feeding for 14 days depressed body weight (Table 1). Thyroid weight was increased from 5 to 7 times that of the control by the feeding of goitrogens. Goitrogenic effect of 0.03 per cent PTU feeding was not exactly the same as goitrin 0.05 per cent feeding though the difference in thyroid weight between the two groups was insignificant.

The changes of thyroidal ^{131}I uptake after the injection of radioiodine are illustrated in Fig. 1. It is clear that the thyroidal ^{131}I uptake was elevated 2 to 3 times that of the control at 0.5 hour after the dosage in goitrogen-fed chicks. Also, the difference between the goitrogen-fed and the control group became increasingly obvious from 1 hour after dosage. The thyroidal ^{131}I uptake of PTU-fed chicks was radically depressed after 1 hour and the incorporated radioiodine in the gland almost vanished from the gland at 24 hours after dosage. On the other hand, the thyroidal ^{131}I uptake of goitrin-fed chicks increased maximum value (36 per cent) being obtained at 8 hours after dosage. The thyroidal ^{131}I uptake at 24 hours after dosage was about 3 times that of the control.

Excretion of ^{131}I (total of urine and feces) for 4 hours after injection of ^{131}I was intensively increased in PTU-fed chicks (about 60 per cent) and promptly increased to over 80 per cent at 24 hours after dosage (Table 2). In the control

TABLE 1. *The Effect of Goitrogens upon Thyroid Weight in 14-Day Old Chicks*

Treatment	Body weight (g)	Thyroid weight (mg/100 g body weight)
Control	135±18(25)	7.7±1.0(10)
Goitrin 0.05%	119±15(24)	39.9±8.5(10)
PTU 0.05%	122± 9(25)	50.8±7.8(10)
PTU 0.03%	125±11(23)	45.2±8.1(10)

±Standard deviation of the mean.
Figures in parenthesis indicate number of chicks.

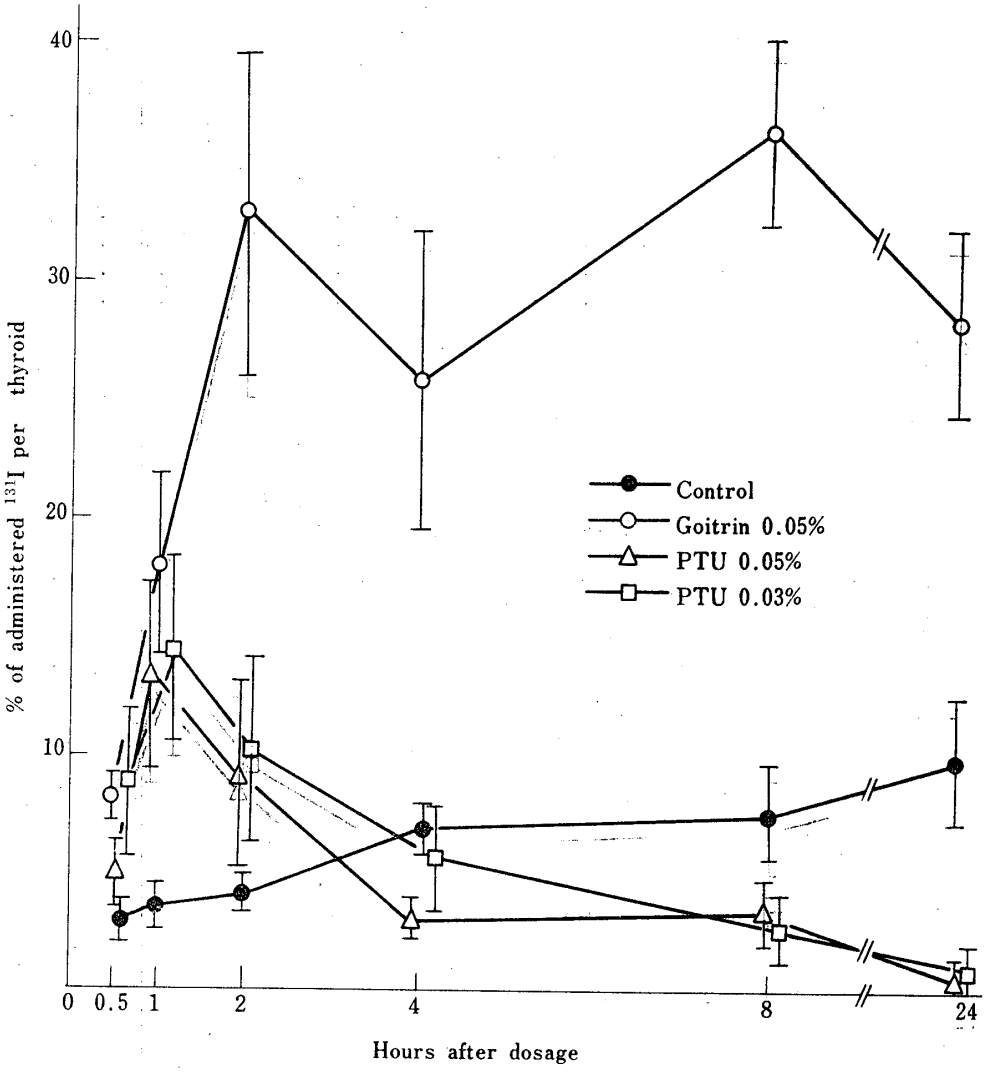

FIG. 1. The changes of thyroidal uptake of ^{131}I after injection of radioidodine. Each point represents an average value for 5 animals. The vertical bars represent the standard deviation of the mean.

TABLE 2. *The Effect of Goitrogen upon the Excretion of ^{131}I after Administration of Radioiodine in* **14***-Day Old Chicks*

Treatment	Hours after dosage		
	4	8	24
	(% of administered ^{131}I per bird)		
Control	53.2	64.2	66.3
Goitrin 0.05%	27.5	31.8	34.0
PTU 0.05%	58.8	81.4	85.6
PTU 0.03%	58.9	73.7	80.2

TABLE 3. *The Effect of Goitrogen upon the Plasma per cent $PB^{131}I$ in* **14***-Day Old Chicks*

Treatment	Hours after dosage		
	4	8	24
	(% of plasma total ^{131}I)		
Control	0.9±0.3	6.3±2.4	52.5±3.9
Goitrin 0.05%	1.4±0.8	13.0±2.6	35.7±7.6
PTU 0.05%	0.5±0.2	0.7±0.3	7.1±6.6
PTU 0.03%	0.4±0.2	1.5±1.0	7.9±6.6

chicks, half of the injected ^{131}I was excreted within 4 hours. The excretion of ^{131}I in the goitrin-fed group, however, was half that of the control and PTU-fed groups at 4 hours and also half that of the cntrol at 24 hours after dosage.

The plasma per cent $PB^{131}I$ in all groups were 0.4 to 1.4 per cent within 4 hours after dosage and thereafter they were increased. The plasma per cent $PB^{131}I$ in goitrin-fed chicks was twice that of the control at 8 hours and depressed to 68 per cent of the control at 24 hours after dosage. In PTU-fed chicks plasma per cent $PB^{131}I$ was 15 per cent that of the control.

The changes of percentage in $^{131}I^-$ iodide (I^-) for total thyroidal ^{131}I in the gland are illustrated in Fig. 2A. In the control chicks, the percentage of I^- is in relatively high level in the early stage of radioiodine injection and reaches an equilibrium state of 8 per cent at 2 hours after dosage. In the 0.05 per cent PTU-fed chicks an increased percentage of I^- (40 to 60 per cent) was obtained from 0.5 hours after dosage and continued for 8 hours. The percentage of I^- in the 0.03 per cent PTU-fed group was also increased, but remained lower than that of the 0.05 per cent PTU-fed chicks. On the other hand, the percentage of I^- in the 0.05 per cent goitrin-fed chicks was not elevated even at the early phase after ^{131}I injection (0.5 hour after dosage) and a lower percentage of I^- in the goitrin-fed group than in those of the control or the PTU-fed group was hept throughout.

Ratios of ^{131}I-iodotyrosines (monoiodotyrosine, MIT and diiodotyrosine, DIT) per $^{131}I^-$ in the thyroid lobes of the control group were at levels of from 5 to 8 in the

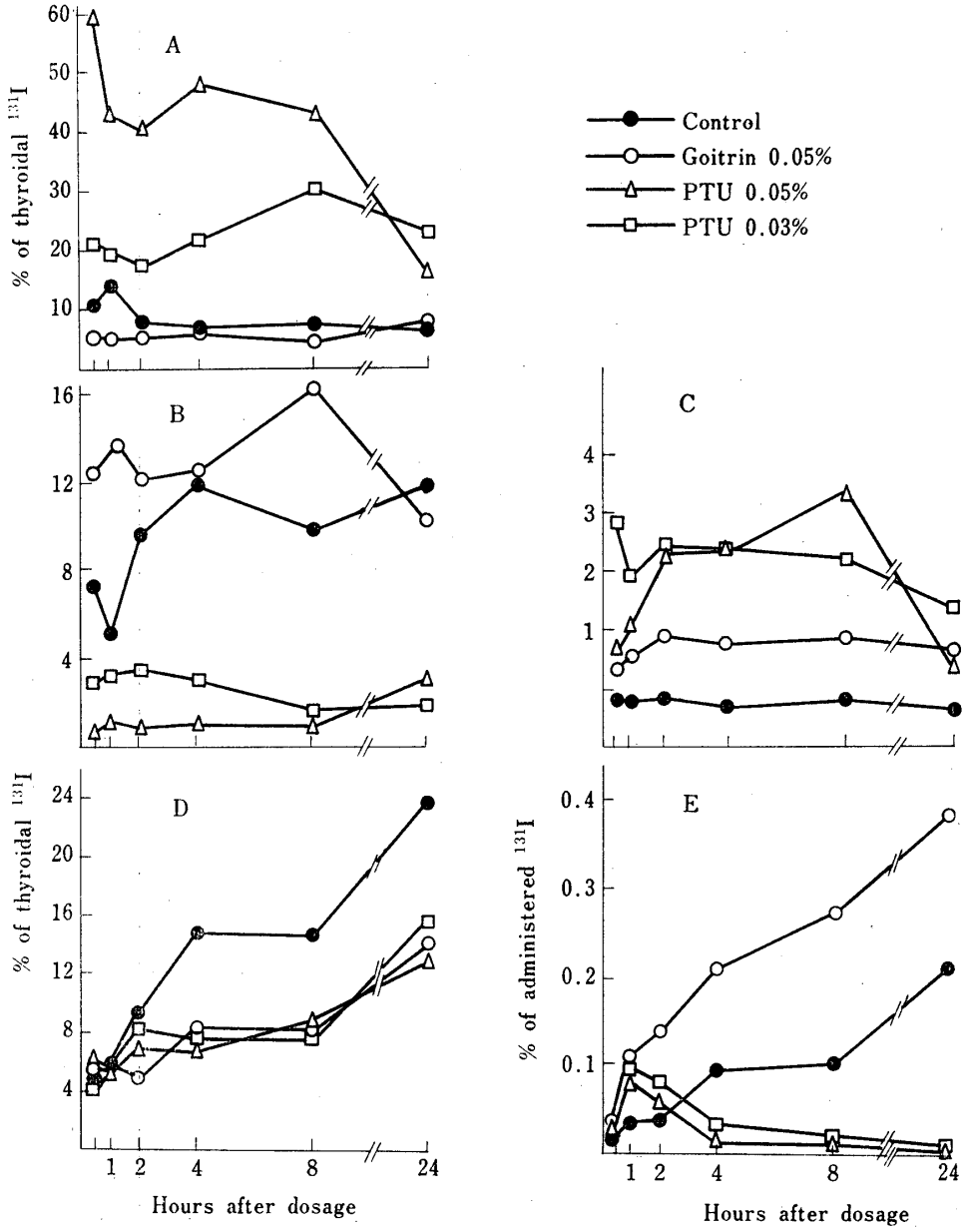

FIG. 2. The changes of percentage or ratio of radioactive iodinated substances in the thyroid lobes. (A) Percentages of I^- for total thyroidal ^{131}I. (B) Iodotyrosine/I^- ratios. (C) MIT/DIT ratios. (D) Percentages of iodothyronines for total thyroidal ^{131}I. (E) Amounts of iodothyronines in the thyroid gland (percentages of iodothyronines for dosage of ^{131}I.)

early period after injection of ^{131}I and increased to a level of 10–12 (Fig. 2B). This seems to indicate that the oxidation ability of iodide is at a low level in the early period after ^{131}I administration. An apparent lower value in ratio of iodotyrosines per I^- was shown in 0.05 per cent and 0.03 per cent PTU-fed chicks immediately after injection of ^{131}I and throughout. It is estimated in goitrin-fed chicks that ratio of iodotyroisines per I^- was not depressed and that a higher value was observ-

ed in the early phase after the dosage of ^{131}I differing from that of PTU-fed chicks.

The ratio of ^{131}I-monoiodotyrosine and ^{131}I-diiodotyrosine (MIT/DIT) in the control chicks was about 0.8 throughout, from 0.5 hour after dosage (Fig. 2C). The most elevated MIT/DIT ratio was demonstrated in both PTU-fed chick groups. Similarly increased MIT/DIT ratio was demonstrated in goitrin-fed chicks but it was lower than that of PTU-fed chicks.

The changes of percentage of ^{131}I-iodothyronine (triiodothyronine, T_3 and thyroxine, T_4) are given in Fig. 2D. No significant difference was found between the 4 groups in synthesis of iodothyronines in the thyroid gland until 1 hour after dosage and about 4 to 6 per cent of the newly synthesized iodothyronines was obtained in each group. The synthesis of iodothyronines in the thyroid of the control chicks showed a rapid increase to the 15 per cent level by 4 hours and thereafter reached to level of 23 per cent in 24 hours after dosage which was almost the equilibrated state of metabolism of injected ^{131}I in the thyroid gland. In the thyroid of the PTU-fed chicks, the synthetic ability of the iodothyronines was depressed from 2 hours after dosage and then no increment of synthesis of iodothyronines was demonstrated. Also, a depression of the thyroidal iodothyronines synthetic ability was given 2 hours after dosage in the goitrin-fed chicks. The synthetic activity of the iodothyronines in goitrogen-fed chicks is about a half of the control at 8 hours and 2/3 of the control at 24 hours after dosage.

The amount of ^{131}I-iodothyronines synthesized in the thyroid gland was demonstrated as percentage of total amount of administered ^{131}I in Fig. 2E. The amount of iodothyronines synthesized is about 0.01 to 0.05 per cent in all groups at 0.5 hours and then increased to the 0.1 per cent level at 1 hour after dosage in goitrogen-fed chicks. Then the amount of iodothyronines decreased rapidly and the iodothyronines almost vanished by 24 hours afer dosage in both PTU-fed groups. In control chicks, the amount of iodothyronines increased gradually to 0.1 per cent at 8 hours and 0.2 per cent at 24 hours after dosage. The most striking feature in goitrin-fed chicks and the obviously different feature from both control and PTU-fed chicks was the intensively increased amount of iodothyronines in the thyroid after 2 hours. The amount of iodothyronines synthesized in goitrin-fed chicks is about two times that of the control 24 hours after injection.

Discussion

Present data shows that the goitrogenic effect (hypertrophy of thyroid) of 0.03 per cent PTU feeding is not exactly the same as 0.05 per cent goitrin feeding. However, goitrogenic activity of 0.05 per cent goitrin is 0.9 times that of 0.03 per cent PTU and is less than that of 0.05 per cent PTU. Also, there is no significant difference between that of the 0.05 per cent goitrin and the 0.03 per cent PTU. It seems that the effect of goitrin may be compared with that of 0.03 per cent PTU, which have almost equal goitrogenic activity.

Thyroidal enlargement (6 to 7 times that of the control), rapid release of ^{131}I incorporated in the thyroid gland and extremely rapid excretion of ^{131}I were obtained in chicks fed 0.05 and 0.03 per cent PTU. On the other hand, thyroid hypertorphy (5 times that of the control), high uptake of ^{131}I in the gland and depressed excretion of ^{131}I were indicated in chicks fed 0.05 per cent goitrin. These results are in good agreement with the data in our previous reports (1, 2). However, this increased thyroidal radioiodine uptake was never reported though depressed thyroidal ^{131}I uptake was obtained by feeding of goitrin to rats for 20 day (4) and 10 day (5) periods.

It is reported by Rosenberg et al. (6) that the percentage of iodide-^{131}I in components in the labeled thyroid lobes is decreased rapidly immeiately after dosage and reached a constant level (below 10 per cent) within 20 min. in cockerels on iodine supplemented diet. In the present study on chicks, however, the percentage of I^- is relatively high within 1 hour after dosage and thereafter the equilibrated state was shown. It is confirmed in PTU-fed chicks that the oxidation of iodide and the iodotyrosine synthesis from iodide in the thyroid lobes was depressed from the early phase after injection of radioiodine, and that typically marked depression was found in chicks fed 0.05 per cent PTU as is evident from the extensive increase in percentage of I^- and the depression in the ratio of iodotyrosines per I^- (Fig. 2A and 2B). On the other hand, following results were obtained in goitrin-fed chicks; the percentage of I^- is lower than that of the control within 1 hour after dosage and the ratio of iodotyrosines per I^- is higher than that of the control from immediately after the injection of ^{131}I. It suggests that oxidation of iodide and monoiodination of tyrosine is not depressed and preferably is activated by 14 days feeding of 0.05 per cent goitrin.

Elevation of $M^{131}IT/D^{131}IT$ ratio in the thyroid lobes in PTU-fed and goitrin-fed chicks (Fig. 2C) indicate that the biosynthesis of the thryoid hormone was inhibited by the feeding of those agents. The inhibitory effect of goitrin on biosynthesis seems to be smaller than that of PTU.

The antithyroid effects of PTU on the intrathyroidal metabolism of radioiodine in the present study of chicks are in good agreement with the reports on rats (7, 8, 9). Otherwise, high thyroidal uptake of ^{131}I obtained by goitrin administration to chicks seems to be similar to high radioiodine uptake goiter which results from a small dose of PTU or methylthiouracil (MTU) in rats (8, 10, 11) and increase of iodotyrosines synthesis from iodide obtained by goitrin feeding to chicks, to increased monoiodination of tyrosine as a production of other iodinated amino acids is being reduced by administration of small dose of PTU in rats (10, 12). However, distinct differences are pointed out between the effects of small doses of PTU or MTU in rats and that of goitrin in chicks mentioned above that goitrin used in our study produced extrmely larger goiter (5 times the thyroid weight of the control chicks) than that (1 to 1.3 times) obtained by small doses of PTU or

MTU to rats and a smaller I^- level in distribution of iodinated substances in the thyroid lobes was obtained in goitrin-fed chicks, different from PTU-administered rats, as previously stated by Matsumoto et al. (2). From the two points of views described above, it seems to be quite right to consider that the effects of goitrin in chicks is different from that of small doses of PTU or MTU in rats.

The synthetic activity of iodothyronines (T_3 and T_4) within 1 hour after injection of ^{131}I is almost the same (about 4 to 7 per cent) in all groups (Fig. 2D). Thereafter, however the synthesis of iodothyronines from newly incorporated ^{131}I increased in the control chicks. The synthesis of iodothyronines was markedly depressed by the feeding of goitrogens. The depression of the synthetic ability in chicks fed goitrin is almost the same as that in chicks fed PTU, though the mechanism of depression in the thyroid iodine metabolism is not identical in the two groups. It is considered that the depressed synthesis of iodothyronines originates in the inhibition of oxidation of iodide, iodination of tyrosine and synthesis of iodothyronines from iodotyrosines in PTU-fed chicks, and on the other hand mainly in the inhibition of iodothyronine synthesis from iodotyrosines, without the inhibition of the oxidation of iodide, in the goitrin-fed chicks.

The amount of iodothyronines-^{131}I synthesized in the gland is markedly depressed by the feeding of PTU (Fig. 2E). It should be emphasized in the present study that the amount of iodothyronines-^{131}I in the gland is markedly increased (2 times of the control) in goitrin-fed chicks despite inhibition of the synthetic step of thyroid hormone.

It is reestablished that the plasma hormone level is depressed though there is a large amount of thyroid hormone in the gland in goitrin-fed chicks. It is, therefore, presumed that the release and secretion of ^{131}I from the thyroid gland into the circulation is inhibited by goitrin feeding. Particularly, it is presumed that the secretion of thyroid hormone from the gland by hydrolysis of thyroglobulin is depressed or that there is no increment of hydrolysis of thyroglobulin by the increased secretion of TSH from the pituitary caused by the feeding of goitrogen, goitrin. It is well known that TSH and antithyroid drugs enhance the hydrolysis of thyroglobulin and lead to an increase of thyroid hormone secretion (13, 14, 15). It is evident that the effect of goitrin is different from that of PTU on the biosynthesis of thyroid hormone and on the secretion of thyroid hormone from the gland.

References

1) Matsumoto, T., Itoh, H., and Akiba, Y., *Poultry Sci.*, **47**, 1323 (1968)
2) Matsumoto, T., Itoh, H., and Akiba, Y., *Poultry Sci.*, **48**, 1061 (1969)
3) Astwood, E.B., Greer, M.A., and Ettlinger, M.G., *J. Biol. Chem.*, **181**, 121 (1949)
4) Langer, P., *Physiol. Bohemslov.*, **15**, 162 (1966)
5) Faiman, C., Ryan, R.J., Eichel, H.J., *Endocrinol.*, **81**, 88 (1967)
6) Rosenberg, L.L., Goldman, M., La Roche, G., and Dimik, M.K., *Endocrinol.*, **74**, 212 (1964)

7) Richard, J.B., and Ingbar, S.H., *Endocrinol.*, **65**, 198 (1959)
8) Slingerland, D.W., Graham, D.E., Josephs, R.K., Mulvery, Jr., P.F., Trakas, A.P., and Yamazaki, E., *Endocrinol.*, **65**, 178 (1959)
9) Mayberry, W.E., and Astwood, E.B., *J. Biol. Chem.*, **235**, 2977 (1960)
10) Greer, M.A., Whallon, J., Yamada, T., and Iino, S., *Endocrinol.*, **70**, 650 (1962)
11) Yamada, T., and Shichijo, K., *Endocrinol.*, **70**, 314 (1962)
12) Iino, S., Yamada, T., and Greer, M.A., *Endocrinol.*, **68**, 582 (1961)
13) Dzieman, A.J., *J. Cell. Comp. Physiol.*, **21**, 339 (1943)
14) Watson, P., and Trikojus, V.M., *Ann. Chem.*, **607**, 215 (1957)
15) Loughlin, R.E., McQuillan, M.T., and Trikojus, V.M., *Endocrinol.*, **66**, 773 (1960)