# INCREASED RESPONSE ON THE CHICKEN COMB TO ANDROGENS AFTER COMBINED ADMINISTRATION OF THYROID EXTRACT AND THEOPHYLLINE

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Thyroid extract potentiates while methylthiouracil inhibits the response of the chicken comb to testosterone propionate. Combined administration of thyroid extract and theophylline evokes the maximal response to androgens. Theophylline does not potentiate the response of the comb when methylthiouracil is given, but rather reduces it. It is postulated that cyclic adenosine-3',5'-monophosphate plays a role in the potentiation of the response of the comb to androgens by thyroid extract, whether alone or in conjunction with theophylline.

It can now be taken as established that the action of many hormones is effected indirectly through an intracellular mediator, cyclic adenosine-3',5'-monophosphate (CAMP), which is synthesized from ATP by the action of the enzyme adenylcyclase located in the cell membrane [1, 7, 12]. The enzyme phosphodiesterase inactivates CAMP and converts it into inactive adenosine-5'-monophosphate. Theophylline has been shown to inhibit the action of phosphodiesterase, and thereby to potentiate and simulate a number of hormonal effects. Because of this property theophylline has been used to demonstrate the participation of CAMP in various hormonal reactions.

Investigations have shown that the response of the chicken comb to endogenous and exogenous androgens is inhibited in the absence of thyroid hormone [2-4, 11] and potentiated by administration of thyroxine [4, 5]. Thyroid extract, if given together with methylthiouracil (MTU), not only abolishes the inhibitory effect of the inhibitor of thyroid function on the response to androgens, but potentiates this response [2]. It has been concluded that thyroid hormone is essential for the normal response of the comb to androgen [4]. However, the mechanism of this effect of thyroid hormone on the response of the comb to androgens has not been discovered [3].

The object of the investigation described below was to study the role of CAMP in the mechanism of the effect of thyroid hormone on the response of the comb to androgens. The action of theophylline on the comb response and on the ability of thyroid extract to potentiate, and of MTU to inhibit this response, was also studied.*

## EXPERIMENTAL METHOD

Experiments were carried out on 5-day chickens of the Russian White breed. Altogether five series of experiments were performed on 414 chickens. The effect of theophylline, thyroid extract, MTU, and theophylline in conjunction with thyroid extract or MTU on the response of the combs to testosterone propionate was studied. The androgen was injected intramuscularly once a day in a dose of 100 μg for 9 or 10 days. An aqueous solution of theophylline was injected into the crop three times a day in a daily dose of

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TABLE 1. Effect of Theophylline, Thyroid Extract, and a Combination of Both on the Response of Chicken Combs to Testosterone Propionate

| Experiment No. | Group No. | Nature of treatment | No of chickens | Mean weight of comb | |
|---|---|---|---|---|---|
| | | | | in mg | in mg/100 g body weight |
| | 1 | No treatment | 17 | 7,9±0,5 | 20,4±1,2 |
| I | 2 | Theophylline | 16 | 10,5±0,9 $P_{1,2}<0,001$ | 24,9±2,2 $P_{1,2}>0,05$ |
| | 3 | Androgen 9 days | 16 | 98,5±7,7 | 236,0±12,8 |
| | 4 | Androgen + theophylline | 16 | 117,5±11,6 $P_{3,4}>0,05$ | 281,1±20,0 $P_{3,4}>0,05$ |
| | 5 | Androgen + thyroid extract 9 days | 15 | 132,1±9,6 $P_{3,5}<0,01$ | 316,8±23,5 $P_{3,5}<0,01$ |
| | 6 | Androgen + thyroid extract from theophylline 9 days | 10 | 213,1±2,2 $P_{5,6}<0,001$ | 491,0±72,5 $P_{5,6}<0,05$ |
| | 1 | No treatment | 23 | 10,96±0,8 | 31,6±2,5 |
| II | 2 | Androgen 9 days | 19 | 186,4±13,1 | 483,1±37,6 |
| | 3 | Androgen + thyroid extract | 28 | 216,5±22,6 $P_{2,3}>0,05$ | 580,5±62,0 $P_{2,3}>0,05$ |
| | 4 | Androgen + thyroid extract from theophylline 9 days | 27 | 308,9±24,0 $P_{3,4}<0,01$ | 858,0±59,0 $P_{3,4}<0,01$ |
| | 1 | No treatment | 20 | 17,8±0,9 | 31,2±1,4 |
| III | 2 | Androgen 9 days | 19 | 188,3±11,3 | 308,7±18,8 |
| | 3 | Androgen + theophylline 9 days | 22 | 197,7±16,1 $P_{2,3}>0,05$ | 340,0±29,3 $P_{2,3}>0,001$ |
| | 4 | Thyroid extract 16 days, with addition of androgen starting on 8th day | 20 | 253,0±16,9 $P_{2,4}<0,001$ | 443,0±23,3 $P_{2,4}<0,001$ |
| | 5 | Thyroid extract 16 days, with addition of androgen + theophylline starting on 8th day | 20 | 337,9±24,8 $P_{4,5}<0,01$ | 637,5±43,6 $P_{4,5}<0,001$ |

3–4 mg per chicken, also for 9–10 days. Thyroid extract was injected into the crop twice a day in a daily dose of 10 mg, and MTU was given twice a day in a dose of 8–10 mg per chicken for 9, 16, or 19 days. At the end of the experiment the chicken combs were excised at the base and weighed.

In one experiment the hyaluronic acid content in the combs was investigated viscosimetrically. The combs from the chickens of each group were pooled, dehydrated, and defatted with anhydrous acetone, and weighed samples of them (150 mg) were then extracted with 10 ml distilled water on a boiling water bath for 1 h. The extracts were centrifuged and examined viscosimetrically. The viscosity of the extracts obtained reflected mainly their content of hyaluronic acid, i.e., to some extent they reflected also the response of the combs to the androgen [6, 9].

The structure and results of the individual experiments are presented in Tables 1 and 2.

## EXPERIMENTAL RESULTS

The results in Table 1 show that administration of theophylline caused a small increase in the absolute weight of the combs (groups 1 and 2) and a small but not statistically significant increase in the response of the combs to testosterone propionate (experiment I, groups 3 and 4). In all three series of experiments thyroid extracts potentiated the response to androgens as in the writer's previous experiments (in two experiments this increase was statistically significant). Theophylline greatly potentiated this action of thyroid (experiment I, groups 5 and 6; experiment II, groups 3 and 4, and experiment III, groups 4 and 5). The viscosity of the extracts of the combs of the chickens receiving androgen was 1.2, of those receiving androgen with theophylline 1.20, and of those receiving androgen and thyroid extract 1.39. The viscosity of the extracts of combs from chickens receiving androgen together with theophylline and thyroid extract was the highest of all (1.70), indicating an increase in their hyaluronic acid content, i.e., a stronger response of the chicken combs to testosterone propionate.

TABLE 2. Effect of Theophylline on Response of Comb of Hypothyroid Chickens to Testosterone Propionate

| Experiment No. | Group No. | Nature of treatment | Number of chickens | Mean weight of comb | |
|---|---|---|---|---|---|
| | | | | in mg | in mg/100 g body weight |
| | 1 | No treatment | 17 | 15,8±1,8 | 27,7±2,2 |
| I | 2 | Androgen 9 days | 19 | 173,6±17,5 | 310±23,2 |
| | 3 | MTU 16 days, with additional androgen starting on 11th day | 28 | 43,2±8,1 | 73,2±14,8 |
| | | | | $P_{2,3}<0,001$ | $P_{2,3}<0,001$ |
| | 4 | MTU 19 days, with additional androgen + theophylline starting on 11th day | 21 | 39,6±5,2 | 70,7±10 |
| | | | | $P_{3,4}>0,05$ | $P_{3,4}>0,05$ |
| II | 1 | No treatment | 20 | 17,8±0,9 | 31,2±1,4 |
| | 2 | Androgen 9 days | 19 | 188,3±11,3 | 308,7±18,8 |
| | 3 | MTU 16 days, with additional androgen starting on 8th day | 20 | 74,5±12,0 | 124,2±18,3 |
| | | | | $P_{2,3}<0,001$ | $P_{2,3}<0,001$ |
| | 4 | MTU 16 days, with additional androgen + theophylline starting on 8th day | 21 | 43,1±4,2 | 79,8±9,5 |
| | | | | $P_{3,4}<0,05$ | $P_{3,4}<0,05$ |

During administration of MTU, inhibiting thyroid function, the response of the chicken combs to testosterone propionate was considerably weakened (Table 2), in confirmation of earlier findings. Additional administration of theophylline to the hypothyroid chickens not only did not potentiate their response to androgens, but in experiment II it weakened it still further (compare groups 3 and 4). Theophylline thus stimulated the response to androgens only in the presence of an excess of thyroid hormone.

These experiments with theophylline suggest that potentiation of the comb response to androgens by thyroid hormone may be due to participation and mobilization of CAMP. By inhibiting phosphodiesterase, theophylline thereby facilitated the accumulation of CAMP, which led as a result to potentiation of the thyroid effect. If this hypothesis is true, the inhibitory action of MTU on the chicken comb response to androgens can be explained by a disturbance of CAMP synthesis in the comb in the absence of thyroid hormone, with resulting inhibition of the development of the response to androgens.

This suggested explanation of the phenomenon of potentiation of the chicken comb response to androgens described in this paper could be tested by determining the CAMP content in the chicken comb directly under appropriate experimental conditions.

## LITERATURE CITED


1. O. A. Virovets, Uspekhi Sovr. Biol., 65, 384 (1968).
2. P. A. Vunder, Probl. Éndokrinol., No. 4, 30 (1957).
3. P. A. Vunder, Probl. Éndokrinol., No. 3, 34 (1963).
4. P. A. Vunder, A. D. Gorbacheva, V. P. Ermolin, et al., Byull. Éksperim. Biol. i Med., No. 8, 93 (1969).
5. B. B. Blivaiss, Physiol. Zool., 20, 67 (1947).
6. N. F. Boas, J. Biol. Chem., 181, 573 (1949).
7. R. W. Butcher, New Engl. J. Med., 279, 1378 (1968).
8. R. W. Butcher and E. W. Sutherland, J. Biol. Chem., 237, 1244 (1962).
9. A. W. Ludwig and N. F. Boas, Endrocrinology, 46, 291 (1950).
10. R. E. Mancini, D. Brandes, A. Portela, et al., Endocrinology, 67, 430 (1960).
11. D. M. Morris, Endocrinology, 48, 257 (1951).
12. E. W. Sutherland, G. A. Robison, and R. W. Butcher, Circulation, 37, 279 (1968).