

EFFECT OF THYROID EXTRACT COMBINED  
WITH THEOPHYLLINE ON COMB GROWTH  
IN CHICKENS

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Thyroid extract, especially in conjunction with theophylline, intensifies the response of the comb to androgen considerably and maintains comb growth in chickens after the action of the hormone has ceased. The two substances likewise have no effect on the weight of the combs of infantile chickens, whether cyproterone acetate is given additionally or not. Thyroid hormone combined with theophylline exerts its effect on comb growth only in the presence of endogenous or exogenous androgen.

The response of the comb to androgen has been shown to be potentiated after administration of thyroxine [5] and thyroid extract [1-3] and to be inhibited in the absence of thyroid hormone [1-4, 6]. Theophylline considerably potentiates the positive action of thyroid on the response of the comb to androgen [4].

The problem arose whether thyroid hormone, especially in conjunction with theophylline, can stimulate growth of the comb independently of androgen. To answer this question experiments were carried out to study the effect of thyroid extract and theophylline on comb growth in infantile chickens and chickens receiving daily injections of androgen. The students V. I. Ivanov and T. A. Kostyukova assisted with the experiments.

EXPERIMENTAL METHOD

Experiments were carried out on Russian White chickens aged 5 days. Growth of the comb was stimulated by intramuscular injections of an oily solution of testosterone propionate in single daily doses of 100  $\mu$ g for 8-10 days or by dropping an alcoholic solution of testosterone twice a day directly on the comb in a daily dose of 50  $\mu$ g for the same period. An aqueous suspension of thyroid extract was injected into the crop once daily in a dose of 10 mg per bird, and an aqueous suspension of theophylline was injected similarly three times a day in a daily dose of 3-5 mg per bird. Treatment with thyroid and theophylline continued for 8-12 days. The action of endogenous androgen was blocked by subcutaneous injection of an alcohol-oil emulsion of cyproterone acetate,\* twice a day in a dose equivalent to 2 mg per chicken. Observations on restoration of normal comb growth were made for 9-12 days after the action of the androgen had ceased.

The design of the experiments and their results are given in Tables 1 and 2.

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TABLE 1. Effect of Thyroid, Theophylline, and a Combination of Both on Normalization of Comb Weight in Chickens after End of Administration of Androgen ( $M \pm m$ )

Group	Treatment	Number of chickens	Weight of comb		P (for relative weights of combs)
			in mg	in mg/100 g body weight	
1	No treatment	12	10,4 $\pm$ 0,6	21,9 $\pm$ 1,1	
2	Dropping alcoholic solution of androgen on comb for 10 days	11	211,8 $\pm$ 37,4	446,4 $\pm$ 46,1	
3	Administration of androgen for 10 days, then no treatment for 12 days	15	391,3 $\pm$ 33,6	648,9 $\pm$ 45,6	$P_{3,2} < 0,01$
4	Administration of androgen for 10 days, then theophylline for 12 days	23	424,4 $\pm$ 28,4	673,0 $\pm$ 32,6	$P_{4,3} > 0,05$
5	Administration of androgen for 10 days, then thyroid for 12 days	20	480,8 $\pm$ 27,8	814,2 $\pm$ 45,2	$P_{5,3} < 0,01$
6	Administration of androgen for 10 days, then thyroid + theophylline for 12 days	24	552,6 $\pm$ 17,0	981,5 $\pm$ 34,2	$P_{6,3} < 0,01$

TABLE 2. Effect of Thyroid and Thyroid with Theophylline on Restoration of Normal Weight of the Comb in Chickens Receiving Cyproterone Acetate ( $M \pm m$ )

Group	Treatment	Number of chickens	Weight of comb		P (for relative weights of combs)
			in mg	in mg/100 g body weight	
1	No treatment	12	26,1 $\pm$ 4,4	52,3 $\pm$ 7,9	
2	Intramuscular injections of androgen in dose of 100 $\mu$ g daily for 10 days	12	259,8 $\pm$ 26,13	502,3 $\pm$ 42,8	
3	Injections of androgen for first 10 days, then injections of solvent of cyproterone acetate for 9 days	15	283,5 $\pm$ 21,9	419,2 $\pm$ 34,8	$P_{3,2} > 0,05$
4	Injection of androgen for 10 days, then injections of cyproterone acetate for 9 days	17	158,3 $\pm$ 18,3	229,4 $\pm$ 13,9	$P_{4,2} < 0,001$
5	Injection of androgen for 10 days, then injections of cyproterone acetate + theophylline for 9 days	19	164,5 $\pm$ 13,1	245,0 $\pm$ 15,9	$P_{5,4} > 0,05$
6	Injections of androgen for 10 days, then injections of cyproterone acetate + thyroid for 9 days	18	127,5 $\pm$ 9,4	207,7 $\pm$ 14,0	$P_{6,4} > 0,05$
7	Injections of androgen for 10 days, then thyroid, theophylline, and cyproterone acetate for 9 days	24	164,6 $\pm$ 13,7	272,0 $\pm$ 20,5	$P_{7,4} > 0,05$

## EXPERIMENTAL RESULTS

The experiments showed that administration of thyroid in conjunction with theophylline for 8 days to 5-day chickens induced a slight increase in weight of the combs. The relative weight of the comb rose from  $27.5 \pm 1.96$  mg to  $34.3 \pm 4.9$  mg ( $P > 0.05$ ). However, when these substances were given together with an androgen antagonist, which blocked the action of small quantities of endogenous androgen, the weight of the comb was actually reduced (to  $21.7 \pm 1.2$  mg;  $P < 0.05$ ). Meanwhile, thyroid+theophylline considerably potentiated the response of the chickens' combs to injection of testosterone propionate. Whereas the relative weight of the combs of chickens receiving androgen was  $270.4 \pm 15.1$  mg, after the additional administration of thyroid+theophylline the weight of the combs rose to  $538.5 \pm 52$  mg ( $P < 0.001$ ). It could accordingly be concluded that thyroid, in conjunction with theophylline and in the absence of androgen secretion, does not stimulate comb growth in infantile chickens. In the next experiments the effect of thyroid in conjunction with theophylline on the restoration of normal comb growth was studied after the action of the androgen had ceased.

It will be clear from Table 1 that in the 12 days which elapsed after discontinuing local application of testosterone to the comb the weight of this organ continued to rise (groups 2 and 3), although the normal size of the comb was nevertheless restored. This was expressed by curving of the comb, yellowing of its indentations, and disappearance of hyaluronic acid from them (histochemical investigations). Administration of theophylline led to a small increase in weight of the combs, which was not statistically significant. Thyroid, however, led to a significant increase in both the absolute and the relative weight of the combs. Thyroid in conjunction with theophylline produced the greatest weight of these organs (groups 3, 5, and 6); i.e., it led to further growth of the comb despite the discontinued treatment with androgen. These experiments apparently indicate that thyroid in conjunction with theophylline can maintain growth of the comb, once it has begun, in the absence of sex hormone. However, the fact that in the experiment described the comb of control chickens not receiving thyroid and theophylline continued to grow after the local application of androgen had been discontinued suggest that an endogenous source of androgen secretion appeared in the experimental chickens. The caudal part of the comb, as most sensitive to androgen [7], reacted to this secretion of sex hormone, and this led to growth of the comb. Under these conditions and in complete agreement with this previous observations [4], thyroid in conjunction with theophylline potentiated this reaction of the comb. To test this hypothesis an experiment was carried out in which the action of endogenous androgen was blocked by cyproterone acetate after the injections of testosterone propionate had been discontinued (Table 2).

As Table 2 shows, 9 days after the end of androgen administration and the beginning of injections of the solvent of cyproterone acetate, the absolute weight of the chicken comb was unchanged while the relative weight was reduced, although not significantly. In other words, in this experiment also no clear normalization of the comb weight took place, and this is also attributed to manifestation of the action of endogenous androgen whose secretion by the gonads or adrenals had begun to take place. In fact, when injections of cyproterone acetate began after those of testosterone propionate had been discontinued, a considerable increase in weight of the chicken combs ensued. In the presence of the androgen antagonist neither thyroid nor theophylline nor a combination of both inhibited normalization of the comb weight (compared groups 4, 5, 6, and 7); i.e., they did not maintain growth of the comb.

Thyroid hormone, in conjunction with theophylline, can thus stimulate or maintain growth of the comb only in the presence of endogenous or exogenous androgen. That is why the potentiation of comb atrophy in thyroidectomized cocks after castration, described in the literature [6], must be explained by a marked weakening of the action of extragonadal androgen.

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