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Original article

Effects of thyroid state alterations *in ovo* on the plasma levels of thyroid hormones and on the populations of fibers in the plantaris muscle of male and female chickens

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Summary — Propylthiouracil (PTU), thyroxine (T4) or thyreoliberin (TRH) were injected *in ovo* to modify the thyroid state of chicken embryos. Significant sexual differences were observed in the effects of these treatments on the plasma concentrations of thyroid hormones and on plantaris muscle characteristics (DNA, RNA, populations of muscle fibers) in 3- and 35-day old male and female chickens. The T4 plasma concentration is lower in control males; it is decreased in PTU treated females and in the T4 treated females at 35 days. The T3 plasma concentration is lowered at 3 days in all treated chickens and also at 35 days in the TRH treated animals. The slow (STnO) and the fast (FTOG) fibers of the plantaris are always more numerous in males. In controls, the number of FTOG fibers remains steady between 3 and 35 days; at the same time, the number of STnO fibers rises in males only. Both PTU and T4 treatments increase the number of the FTOG and the STnO fibers respectively before and after the 3rd day. TRH treatment increases the number of STnO fibers at 3 and 35 days in males, but reduces it at 3 days in females. Thus changes in the number of FTOG fibers can be induced during *in ovo* myogenesis, whereas the number of STnO fibers may increase after hatching.

chicken / thyroid hormones / muscle fibers / skeletal muscle

Résumé — Effets de modifications *In ovo* de l'état thyroïdien sur les teneurs plasmatiques d'hormones thyroïdiennes et sur les populations de fibres du muscle plantaris chez des poulets mâles et femelles. *Du propylthiouracile (PTU), de la thyroxine (T4) ou de la thyréolibérine (TRH), ont été administrés* in ovo *afin de modifier l'état thyroïdien chez l'embryon de poulet. Ces traitements montrent de fortes différences sexuelles dans leurs effets sur le niveau plasmatique des hormones thyroïdiennes et sur plusieurs caractéristiques (ADN, ARN, populations de fibres) du muscle plantaris chez des poulets de 3 et 35 jours des deux sexes. La concentration plasmatique de T4 est inférieure chez les témoins mâles; elle est abaissée chez les femelles traitées par le PTU et à 35 jours, chez les femelles traitées T4. La concentration plasmatique de T3 est abaissée à 3 jours chez tous les poussins traités et à 35 jours chez les poussins traités TRH. Les fibres musculaires*

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lentes (STnO) et les fibres rapides (FTOG) du plantaris sont toujours plus nombreuses chez les mâles. Chez les témoins entre 3 et 35 jours, le nombre de fibres FTOG ne varie pas alors que le nombre de STnO s'accroît, mais seulement chez les mâles. Les traitements PTU et T4 augmentent le nombre de fibres FTOG et STnO respectivement avant et après le 3e jour. TRH in ovo *accroît le nombre de fibres STnO à 3 et à 35 jours chez les mâles, mais le diminue à 3 jours chez les femelles. Ainsi, des modifications du nombre de fibres FTOG peuvent être induites lors de la myogenèse,* in ovo, *alors que le nombre de fibres STnO peut augmenter après l'éclosion.*

poulet / hormones thyroïdiennes / fibres musculaires / muscle squelettique

INTRODUCTION

The skeletal muscle is made up of multinucleated fibers belonging to 3 main categories. In the terminology of Peter *et al* (1972), they are classified according to their speed of contraction and metabolism: 1), fast-twitch glycolytic fibers (FTG); 2), fast-twitch oxido-glycolytic fibers (FTOG) and slow-twitch oxidative fibers (STO). In birds, STO fibers are present but the slow fibers are mostly slow tonic multiinnervated oxidative fibers, here noted as STnO fibers.

In birds as in mammals, thyroid hormones (TH) contribute to the regulation of the ponderal growth and to the regulation of multiple biological processes. TH play a pivotal role in the differentiation and the maturation of many tissues; in muscle, TH can change the total number of fibers (TNF) and regulate the relative abundance of muscle fibers of various types.

In adult rats, surgical hypothyroidectomy decreases the body weight and lowers the percentage of the fast-twitch fibers in the 4 muscles studied (soleus, plantaris, adductor longus, diaphragma) whereas hyperthyroidism induced by T3 injection decreases body weight but increases the percentage of fast-twitch fibers in the same muscles (Ianuzzo *et al,* 1980).

In the fowl, the *in ovo* administration of methylmercaptoimidazole, an antithyroid drug, increases the number of FTOG fibers in the plantaris and flexor digitorum muscles of adult male chickens (Bacou *et al,* 1980).

Thus, an acute modification of the thyroid state during endocrine system ontogeny eventually modified tissue differentiation and probably modified growth and development.

These results led us to study in 3- and 35-day old male and female chickens the effects of experimental alterations in thyroid state during embryogenesis. We focused on the plasma levels of thyroid hormones, the populations of muscle fibers and the DNA and RNA content of the plantaris muscle, chosen on the basis of its mixed fiber composition (FTOG and STnO).

MATERIALS AND METHODS

NaOH, perchloric acid (PCA) were supplied by Merck; bovine albumin, RNA, DNA, propylthiouracil (PTU), thyroxine (T4), triiodothyronine (T3) were supplied by Sigma, thyreoliberine (TRH) by Bachem, T4 and T3 RIA kits by Bio-Merieux.

We bred our line of New Hampshire CII chickens. Eggs were incubated at 37.8 ± 0.2 °C in a 'La Nationale' forced-draft incubator. The age of the embryos was the time the eggs remained in the incubator. On day 18 of incubation, eggs were placed in the hatchery. After hatching, the chickens were kept in an animal house maintained at 22 °C with infrared heating complement and 12L–12D photoperiod. They were fed *ad libitum* on a corn, wheat and soybean meal based diet (2900 Kcal ME/kg; 19% protein).

Eggs were injected once in the air space on the chorioallantoic membrane with 100 µl of aqueous solutions containing: for PTU chickens: 1 mg of PTU, at day 7 of incubation; for T4 chickens: 100 ng of T4, at day 10 of incubation; for TRH chickens: 1 µg of TRH, at day 10 of incubation.

For injections, the chronology and the doses were chosen according to preliminary experiments where thyroid physiology, hatching levels and effects on the plantaris muscle were considered. We tested 0.5 mg and 1 mg of PTU at day 3 or 7 of incubation, in order to prevent or delay the onset of T4 and T3 secretions. We also performed 1, 2 or 3 100 ng T4 injections between day 6 and 16. In all cases, repeated injections, at 2-day intervals, decreased hatching levels. Higher doses were also unfavourable. The optimum date of injection appeared to be the 10th day. Single injections of 10 ng, 100 ng, 1 µg, 10 µg and 20 µg of TRH were tested at the same age retained for T4; 1 µg was chosen.

Control animals received an injection of 100 µl of sterile water, which as a preceding experiment showed displayed no effect. Each treatment was performed on 4 distinct groups of eggs, including controls; at least 8 animals were used to analyse the muscle characteristics for one sex at one age and for one treatment.

In 3- and 35-day old chickens under ether anaesthesia, a few minutes before sacrifice, heart blood was sampled on heparin and centrifuged for 10 min at 8 000 *g* in an Eppendorf centrifuge. The plasma was stored at –20 °C until the time of T4 and T3 assays.

Both plantaris muscles were cut off and weighed. One muscle was frozen in dry ice and stored at – 20 °C until DNA and RNA analysis. The other was quickly frozen in dry ice-cooled isopentane and stored at –70 °C for histoenzymatic studies.

Muscle fiber typing and measurements

Transverse 12-µm thick serial sections were cut with a cryostat microtome through the upper part of the muscles, just underneath the proximal insertion, in order to count the whole number of fibers. The sections were air-dried at 4 °C and reacted for myofibrillar ATPase after acid preincubation according to the technique of Guth and Samaha (1969), which had been slightly modified by Ashmore *et al* (1978). All quantitative histology was performed using a Visopan Reichert projection microscope.

DNA and RNA assays

The technique of Dainat and Rebiere (1978) was used with slight modifications. All operations were carried out at 0°C. One plantaris muscle was homogenized using an Ultraturrax in 2.5 ml of 0.4 N PCA. The final homogenate was centrifuged at 2 500 *g* for 20 min and the supernatant discarded. The pellet was washed once in 1 ml PCA 0.2 N and the supernatant discarded. The new pellet was dissolved in 2.5 ml 0.3 N NaOH and incubated at 37 °C for 1 h. 0.35 ml 3 N ice cold PCA were added and the samples were maintained on ice for 10 min before centrifugation (2 500 *g* for 20 min). The supernatant was collected and the pellet washed once in 2 ml of 0.4 N PCA. Both supernatants were pooled for RNA determination. The pellet was further dissolved in 2.5 ml 0.3 N NaOH. The solution was made acidic with 0.4 ml 3 N ice-cold PCA and further with 0.7 ml 1 N PCA; the samples were then heated to 70 °C for 15 min and quickly refrigerated in ice for 10 min. The supernatant was kept and the pellet was collected once in 1 ml PCA 0.2 N. Both supernatants were pooled for DNA determination.

RNA and DNA were estimated by spectrophotometric determination, at 260 nm for RNA, using yeast RNA as standard, and at 266 nm for DNA using calf thymus DNA as standard.

T4 and T3 assays

Plasma T4 or T3 assays were carried out simultaneously on control and experimental samples for each stage. Total plasma T4 or T3 concentrations were measured in duplicate by the radioimmunoassay method with T4 or T3 specific antibodies (anti-T3 or anti-T4 rabbit serum) coated on the bottom of test tubes (BioMerieux ^{125}I T4 and ^{125}I T3 coat RIA). The reaction was performed in Tris–NaCl buffer pH 8.6 with 8-anilino-1-naphthalene-sulfonic acid and natrium salicylate to eliminate interference due to serum binding proteins (TBG, TBPA, albumin) and to

allow a direct assay of T4 and T3. Standards were developed using chicken plasma depleted of TH on charcoal dextran. The assays were performed by incubation of 100 µl of plasma at 37 °C for 1 h with 400 µl of ^{125}I T3 or ^{125}I T4 in test tubes coated with anti-T3 or anti-T4 antibodies. After incubation, the solutions were discarded and the test tubes rinsed in 2 ml of water. The radioactivity was measured in a gamma counter. The values were calculated using a logit-log standard curve derived from 5 duplicates of various hormone concentrations. The minimum amount of T3 and T4 significantly differing from the 0 concentration with a probability of 95% was 0.2 nM and 0.5 nM respectively. Percentage of cross-reactivity at B/B0 = 0.5 was 100 for triiodothyronine and 0.15 for L-thyroxine with the T3-kit, and 100 for thyroxine and 4.5 for L-triiodothyronine with the T4 kit.

Statistical analyses

Statistical analyses were performed by 1-way or 2-way ANOVA analysis of variance. The differences in mean values were further analysed using 1-way ANOVA between controls and each treatment of the same sex and the same age, and between ages for each treatment.

RESULTS

In the PTU treated chickens, hatching occurred 3 days later than in controls; there was a 40% range of hatching ability. TRH-treated chickens hatched 0.5 day in advance. The hatching ability and the survival of T4 and TRH treated animals were similar to that of controls.

The plasma levels of T4 and T3 varied respectively from 2.73 to 6.61 ng/ml and from 0.59 to 2.58 ng/ml (fig 1). In controls and in TRH-treated chickens T4 levels were lower in males. PTU and T4 treatments tended to reverse these sexual differences.

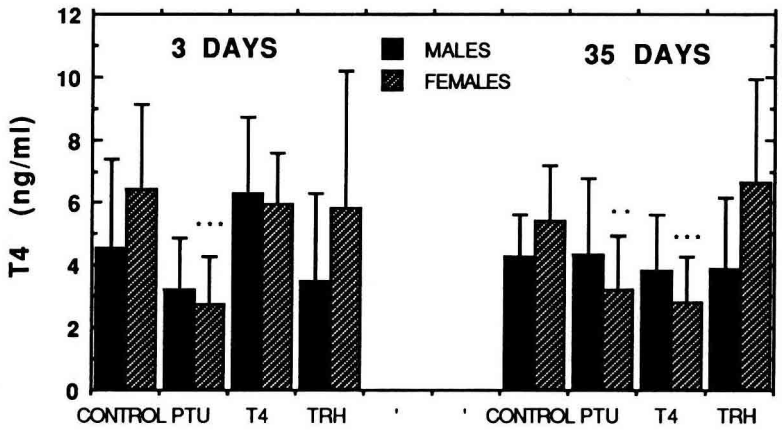
The PTU treatment resulted in a significant lowering of T4 and T3 plasma concentrations in the 3-day old chickens. The T4 treatment lowered the levels of T4 at 35 days and T3 at 3 days. The TRH treatment lowered T3 at 3 and 35 days.

Body weight and muscle plantaris weight (fig 2) were lowered at 3 days in PTU and TRH treated chickens and enhanced at 35 days. The plantaris muscle weight increase seemed related to higher quantities of muscle RNA, especially in TRH treated animals where the values were about twice the control values (fig 3). The RNA/DNA ratio was higher in all treated 35-day old chickens; it was only slightly elevated on the 3rd day by the T4 injection.

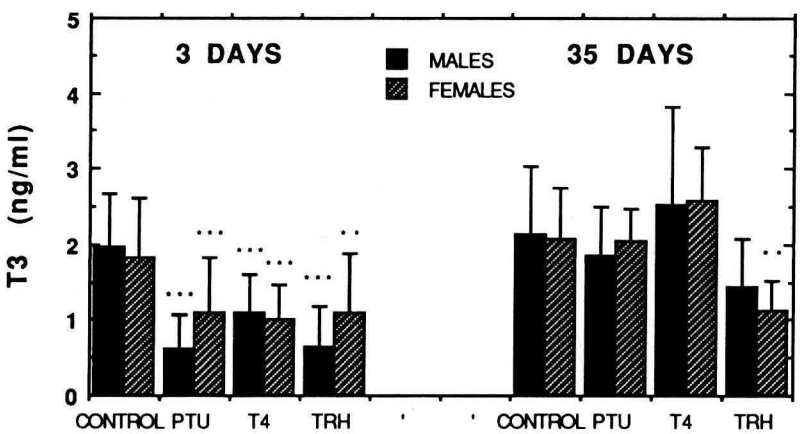
The DNA plantaris muscle content increased ≈ 10-fold between days 3 and 35. The T4 treatment increased the DNA plantaris content at 3 days and decreased it at 35 days (fig 4). TRH treatment increased the DNA plantaris content at 35 days.

In controls and in treated male chickens, the number of slow and fast muscle fibers (fig 5), and consequently the total number of fibers (TNF) (fig 4) were higher than in females. In controls, the TNF remained steady in females, but increased from 3 to 35 days in males due to a preferential increase of the number of slow fibers. All treatments enhanced the TNF at each studied age, except for TRH treated females; the increases in TNF were related to a higher number of fast fibers from the 3rd day on, and to a gradual increase of slow fibers between 3 and 35 days.

The cross-sectional areas of STnO fibers did not differ between males and females and between controls and treated chickens; the mean was 234 ± 56 µm^2 at 3 days and 1162 ± 279 µm^2 at 35 days. The cross-sectional areas of FTOG fibers were higher in females at 3 days, 272 ± 80 µm^2 *versus* 228 ± 54 µm^2. At 35 days, they were slightly higher in PTU treated females (1 683 ± 192 µm^2) as compared to the mean of other male and female chickens (1 429 ± 343 µm^2).

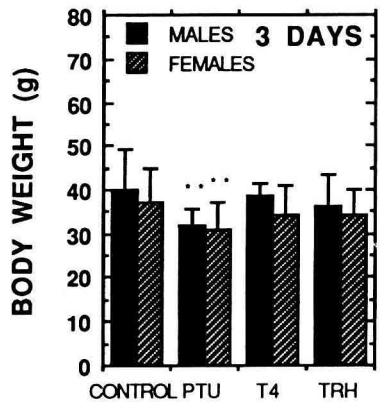


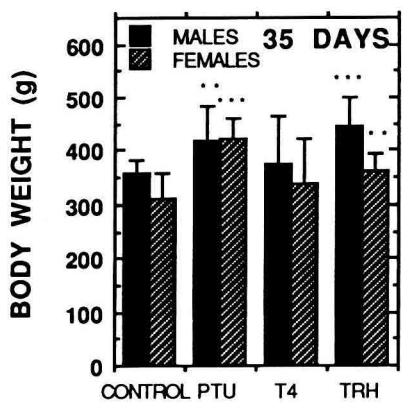
Significance :								
Treatments	-	0.000	0.121	0.464	-	0.015	0.002	0.813
Sexes	0.042	0.092	0.089	0.014	0.029	0.428	0.124	0.004
Interaction	-	0.069	0.085	0.783	-	0.020	0.033	0.120



Significance :								
Treatments	-	0.000	0.000	0.000	-	0.453	0.123	0.001
Sexes	0.783	0.120	0.973	0.113	0.887	0.858	0.787	0.530
Interaction	-	0.094	0.626	0.383	-	0.623	0.982	0.561

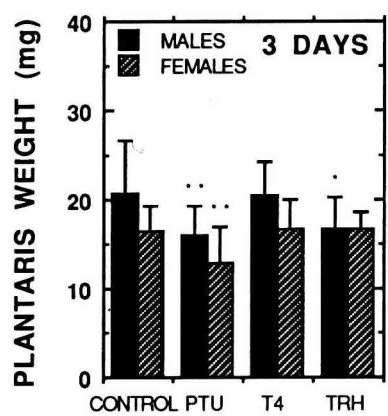
Fig 1. Effects of the administration of PTU, T4 and TRH on the plasma levels of T4 and T3 in male and female chickens at 3 and 35 days. Values are means ± SD. Significance was calculated by variance analysis. 1-way ANOVA: at each age between males and females in controls; between treated and controls for a same sex (*); (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). 2-way ANOVA: between controls and treated animals.

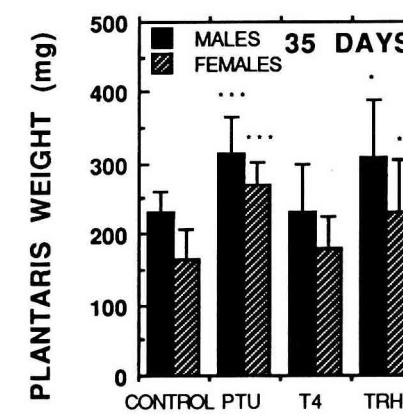




Significance :				
Treatments	-	0.000	0.988	0.116
Sexes	0.101	0.491	0.059	0.090
Interaction	-	0.559	0.431	0.788

-	0.000	0.336	0.000
0.033	0.077	0.085	0.000
-	0.123	0.782	0.127

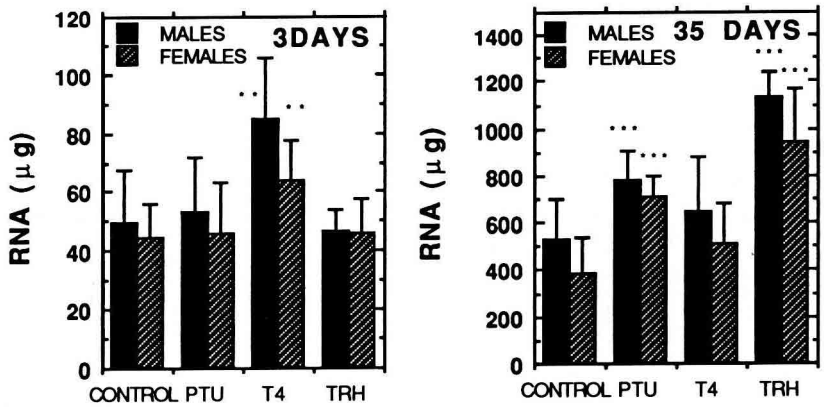




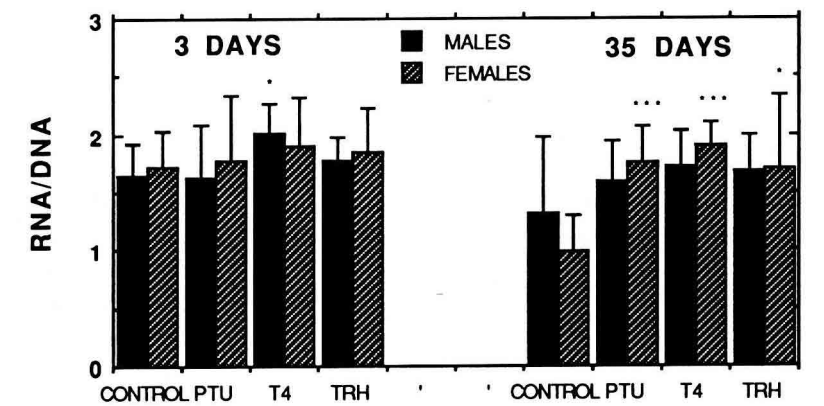
Significance :				
Treatments	-	0.006	0.915	0.042
Sexes	0.023	0.000	0.002	0.083
Interaction	-	0.684	0.985	0.069

-	0.000	0.457	0.001
0.002	0.000	0.001	0.001
-	0,509	0,407	0,749

Fig 2. Body weight and plantaris muscle weight of 3- and 35-day old male and female chickens treated *in ovo* with PTU, T4 or TRH. Values are means ± SD. Significance was calculated by variance analysis. 1-way ANOVA: at each age between males and females in controls; between treated and controls for a same sex (*); (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). 2-way ANOVA: between controls and treated animals.

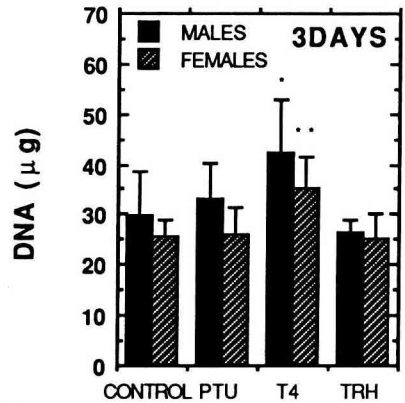


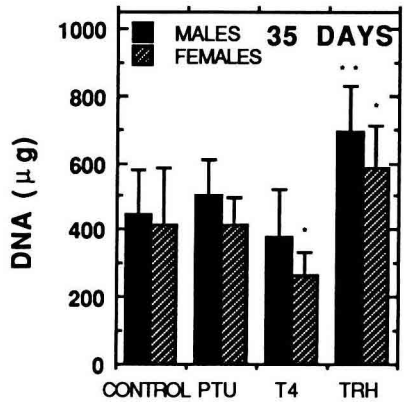
Significance :								
Treatments	-	0.661	0.000	0.900	-	0.000	0.048	0.000
Sexes	0.598	0.125	0.005	0.533	0.147	0.011	0.076	0.008
Interaction	-	0.813	0.137	0.781	-	0.434	0.914	0.796



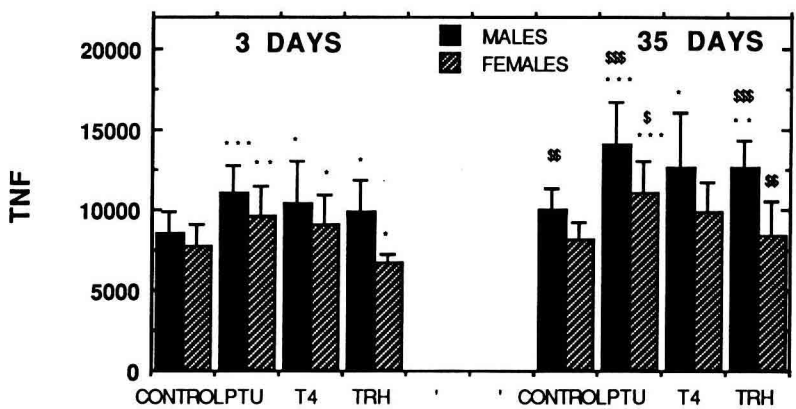
Significance :								
Treatments	-	0.953	0.035	0.370	-	0.020	0.000	0.015
Sexes	0.664	0.384	0.611	0.631	0.179	0.800	0.967	0.444
Interaction	-	0.811	0.480	0.993	-	0.102	0.082	0.408

Fig 3. RNA and RNA/DNA contents of the plantaris muscle in 3- and 35- day old male and female chickens treated *in ovo* with PTU, T4 or TRH. Values are means ± SD. Significance was calculated by variance analysis. 1-way ANOVA: at each age between males and females in controls; between treated and controls for a same sex (*); (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). 2-way ANOVA: between controls and treated animals.



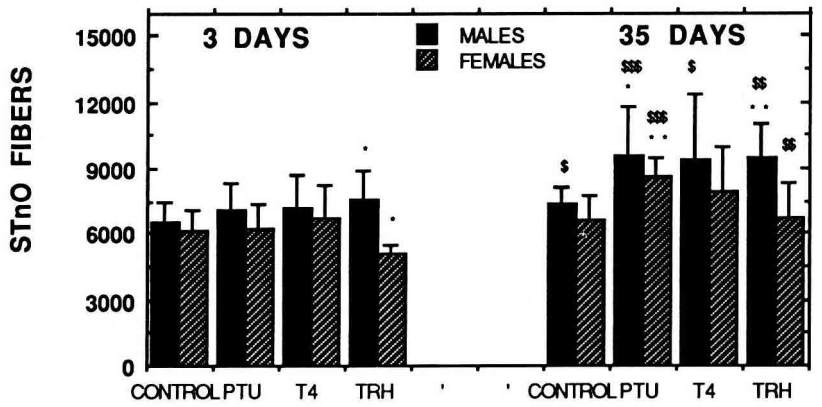


Significance :								
Treatments	-	0,379	0,011	0,471	-	0.467	0.034	0.002
Sexes	0.196	0.002	0.000	0.151	0.758	0.102	0.079	0.144
Interaction	-	0.515	0.577	0.573	-	0.512	0.378	0.509

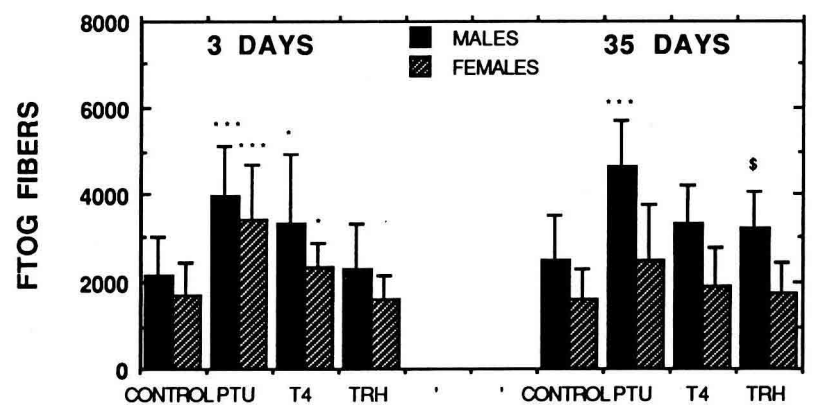


Significance :								
Treatments	-	0.000	0.002	0.714	-	0.000	0.007	0.018
Sexes	0.075	0.002	0.025	0.000	0.005	0.000	0.001	0.000
Interaction	-	0.487	0.577	0.003	-	0.484	0.548	0.039

Fig 4. DNA content and total number of fibers (TNF) in the plantaris muscle of 3- and 35-day old male and female chickens treated *in ovo* with PTU, T4 or TRH. Values are means ± SD. Significance was calculated by variance analysis. 1-way ANOVA: at each age between males and females in controls; between treated and controls for a same sex (*); (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). 2-way ANOVA: between controls and treated animals; between 3 and 35 days for a same sex and treatment ($); (*,$ P < 0.05; **,$$ P < 0.01; ***,$$$ P < 0.001).



Significance :								
Treatments	-	0.118	0.067	0.756	-	0.000	0.016	0.076
Sexes	0.115	0.037	0.124	0.000	0.113	0.026	0.086	0.001
Interaction	-	0.426	0.978	0.000	-	0.851	0.606	0.077



Significance :								
Treatments	-	0.000	0.003	0.810	-	0.000	0.062	0.098
Sexes	0.102	0.009	0.018	0.010	0.050	0.000	0.000	0.000
Interaction	-	0.861	0.369	0.162	-	0.075	0.412	0.108

Fig 5. Numbers of fast (FTOG) and slow (STnO) fibers in the plantaris muscle of 3- and 35-day old male and female chickens treated *in ovo* with PTU, T4 or TRH. Values are means ± SD. Significance was calculated by variance analysis. 1-way ANOVA: at each age between males and females in controls; between treated and controls for a same sex (*); between 3 to 35 days for a same sex and treatment ($); (*,$ $P < 0.05$; **,$$ $P < 0.01$; ***,$$$ $P < 0.001$). 2-way ANOVA: between controls and treated animals.

DISCUSSION

The low hatching values of *in ovo* PTU treated embryos and their delay in hatching are mainly due to a resorption defect of the yolk sac as reported for other antithyroid drugs (Adams and Bull, 1949; Goussopoulos *et al,* 1974). The slight speeding up of hatching time in the TRH treated embryos seems related to an acceleration of the maturation processes.

Plasma levels of T4 and T3

The T4 plasma concentrations measured by RIA in this experiment are lower than in other breeds (Klandorf *et al,* 1978; Hylka *et al,* 1986); they correspond to the values obtained with the ELISA technique (data not shown) and competition assay (Bacou *et al,* 1980). The T3 plasma concentrations equal the values reported for other breeds (Newcomer, 1974; Bobek *et al,* 1977; Klandorf *et al,* 1978; Kühn *et al,* 1982; Hylka *et al,* 1986).

We found a sexual difference in the T4 plasma concentration which was higher in females, as reported by Bacou *et al* (1980). Such sexual difference may correspond to higher peripheral tissue deiodinase activities in males, as measured in chicken muscle (Dainat *et al,* in preparation) or in rat liver and kidney (Harris *et al,* 1979; Coka, 1979). TRH treatment does not change these sexual differences, whereas PTU and T4 treatments tend to inverse them, perhaps by modifying the maturation of the thyroid and of the gonadal axis and/or the activities of the peripheral cellular deiodinase activities.

We also found a sexual difference in the effects of PTU and T4 treatments on plasma T4 concentrations. *In ovo*, PTU as MMI (Bacou *et al,* 1980) induces a persistently low level of T4 which is maintained after hatching in females only until the 35th day. Since T4 plasma concentrations are higher in control females, some kind of interaction between female steroids and T4 secretion may exist, perhaps through progesterone, which peaks *in ovo* (Kalliekaran and Hall, 1974) at the same time as T4; this could also explain the higher peak of T4 in females (Bacou *et al,* 1980). As for other tissues, the hypothyroidism induced *in ovo* by PTU could delay the maturation of the ovary and reduce the progesterone secretion.

As T3 is considered the active hormone, all treatments appear to induce hypothyroidism during the perinatal period. This state evolves to euthyroidism at 35 days in PTU and T4 treated animals but persists in TRH treated females. The lower plasma T3 concentrations could be due to various mechanisms such as a decrease of T3 secretion by the thyroid gland and/or a decrease of tissue T4 5' deiodinase activity.

PTU hypothyroidism is linked to decreased tissue T4 5'deiodinase activities, as observed in rats (Oppenheimer *et al,* 1972; Leonard and Rosenberg, 1978; Silva and Mattews, 1984) and in other species (Geffner *et al,* 1975). *In ovo*, this drug or some metabolite could persist and remain active until hatching when it could be eliminated. T4 and TRH, which are physiologically quickly metabolized into ineffective compounds, likely affect the thyroid state in a different way. We suspect that they may bring about transient hyperthyroidism which could interfere with the maturation of the deiodinase system, lowering the T4 5'-deiodinase activity, whereas T4 and TRH respectively stimulate this activity in adult rat liver and kidney (Grussendorf and Hüfner, 1977; Kaplan and Utiger, 1978; Van Doorn *et al,* 1982; Jennings *et al,* 1984) and in adult chicken liver (Kühn *et al,* 1986). TRH is also able to stimulate growth hormone secretion in immature chickens (Decuypere and Scanes, 1983;

Huybrecht *et al,* 1985; Scanes *et al,* 1987; Harvey, 1990); in chickens this hormone increases the 5' monodeiodinase activity in embryonic and in adult liver (Kühn *et al,* 1986; Darras *et al,* 1990). Otherwise T4 and TRH could have induced a reactional hypothyroidism, but this hypothesis is less probable since hatching occurs within a normal space of time or is even slightly accelerated in TRH treated chickens. In this case the recovery at 35 days is delayed, particularly in females where T4 levels are still within the normal range.

Thus, after hatching our treatments induced decreases in the plasma concentrations of thyroid hormones probably in relationship with alterations in the ontogeny of various hormonal systems. At this stage, the maturation of the thyroid gland must also be affected, but other modifications could be involved which we are now investigating: thyroid hormones levels, hormone metabolism and clearance, tissue deiodinase activities.

Fiber populations in the plantaris muscle

Increases in plantaris total number of muscle fibers (TNF) were ascertained in all treated chickens, with the exception of TRH treated females. These changes correspond to alterations of the *in ovo* myogenesis since they were measured from the 3rd day after hatching, but they also correspond to modifications in the postnatal evolution of TNF. Moreover, the treatments diversely affected the various populations of muscle fibers.

In PTU treated chickens the TNF is greater, as Bacou *et al* (1980) reported in MMI treated males. This could be explained by a precocious effect of hypothyroidism on myogenesis and/or development of the motor neurones as the T3 plasma concentration is low at 3 days. This higher TNF is based upon an increase in both slow and fast fiber numbers. However, a striking difference appears in the effects of PTU treatment on fiber populations: the increase in fast fibers occurs only before hatching, whereas the number of slow fibers increases between hatching and 35 days. This increase is higher in treated males than in controls. In control females, there is no change but in the treated females the slow fiber number increases as much as in treated males.

The post-natal increase of STnO fibers in PTU treated chickens may rely on hyperplasy either of myoblasts still present in the muscle at this stage or of satellite cells, extending myogenesis after hatching. A further conversion of fibers from one type to another could occur during development since in adults treated *in ovo* by MMI, Bacou *et al* (1980) only observed a significant increase in fast fibers, which could be related to a transformation of slow fibers into fast fibers as an adaptation to functional requirements.

As with PTU but to a lesser degree, T4 injected *in ovo* increases the TNF and the numbers of slow and fast fibers, but perinatal myogenesis seems accelerated as FTOG, DNA and RNA values are higher at 3 days, but equal or slightly under normal values at 35 days. The increases in slow and fast fiber numbers may be linked to the transitory perinatal hypothyroidism observed.

TRH administration *in ovo* increases TNF in males at 35 days. As compared to PTU and T4 effects, this is based upon highly different hormonal and tissual evolution and upon strong sex/TRH interferences on myogenesis. At 3 days, STnO fiber numbers of TRH treated chickens are higher in males and lower in females than in controls. In both sexes, the STnO fiber number increases after hatching, reaching

normal values in females. FTOG fiber numbers equal normal values at 3 days, and rise until 35 days in males. This postnatal evolution of both populations of fibers corresponds to dramatic increases in muscle DNA and RNA contents. As the TNF is increased in males within the same range as in other treatments, the cellular units must be more numerous, of lower size, and could correspond to higher growth potential or to the speeding up of some processes. In TRH treated animals, the T3 plasma concentration remains lower at 35 days. Thus, as in other treatments, some degree of hypothyroidism does not inhibit DNA and RNA synthesis, but could even stimulate them in muscle and possibly in other tissues since at 35 days the fiber number increase corresponds to higher plantaris and body weights.

Other mechanisms could be implicated. For example, a "trophic effect" of TRH on fetal rat spinal motor neurones in cultures has been described (Schmidt-Achert *et al,* 1984). *In vivo,* this could result in a greater survival of motor neurones during embryonic life, and in a related increase in the number of fibers (Ross *et al,* 1987).

Our treatments interfere with myogenesis. They have quantitative rather than qualitative effects. Thus, PTU *in ovo* in the chicken and an experimental hypothyroidism in newborn or in adult rats differently affect the populations of muscle fibers. In newborn rats, PTU administration from birth inhibits the normal increase of slow and fast fiber numbers in the soleus muscle (Sugie and Verity, 1985). But this also induces muscle metabolic disturbances (Nemeth *et al,* 1989) and a marked diminution of body weight and cell multiplication. Since early postnatal protein restriction in rats and malnutrition in mice inhibit the increase of fast and slow fiber numbers (Ihemelandu, 1985; Timson and Dudenhoeffer, 1985), the TNF reduction reported by Sugie and Verity (1985) could be the consequence of hypotrophy in their animals rather than a direct effect of hypothyrodism, whereas acute antenatal treatment of the chickens does not induce hypotrophy or DNA lowering. In adult rats, surgical thyroidectomy does not modify the TNF but lowers the percentage of fast fibers (Ianuzzo *et al,* 1980; Nwoye and Mommaerts, 1981). In fact, thyroid hormones affect the synthesis of the muscle myosin isoforms during development (Butler-Browne *et al,* 1984), regeneration (d'Albis *et al,* 1987), and at the adult stage (Izumo *et al,* 1986). The myogenesis modifications induced during the embryonic and the perinatal period in our chickens are due to other mechanisms.

Our results show that various treatments able to interact with the thyroid stade of the chicken *in ovo* decrease T3 plasma level at 3 days, increase the total number of muscle fibers in the plantaris muscle at 3 and 35 days and change in different manners the ontogeny of the various muscle fiber populations. A study of the perinatal variations of thyroid hormone levels and the development of motor innervation of the plantaris muscle in control and treated animals could contribute towards an explanation of our results.

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