

Review article

Physiological studies on the sex-linked dwarfism of the fowl: a review on the search for the gene's primary effect

M. Tixier-Boichard¹, L.M. Huybrechts², E. Kühn², E. Decuypere³,
J. Charrier⁴ and P. Mongin⁵

¹ Institut National de la Recherche Agronomique, Laboratoire de Génétique Factorielle, 78350 Jouy-en-Josas, France;

² Zoological Institute, Laboratory of Comparative Endocrinology, Naamsestraat 61, 3000 Leuven, Belgium;

³ Laboratory for Physiology of Domestic Animals, Kardinaal Mercierlaan 92, 3030 Heverlee, Belgium;

⁴ Institut National de la Recherche Agronomique, Station de Physiologie de la Croissance, 9 place Viala, 34060 Montpellier, France;

⁵ Institut National de la Recherche Agronomique, Station de Recherches Avicoles, Centre de Recherches de Nouzilly, 37380 Monnaie, France

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Summary — The results obtained in the last 10 years on the endocrinological and metabolic effects of the sex-linked dwarf gene of the fowl are reviewed in order to identify its mode of action on growth and other traits.

Among the main factors involved in growth regulation, thyroid hormones, T₄ and T₃, growth hormone, GH, and its related growth factor, IGF-I, were the most studied in dwarfs. These birds are characterized by low circulating levels of T₃ and IGF-I in spite of normal or even increased levels of T₄ and GH. The T₃ deficiency is explained by a lower peripheral activity of T₄ monodeiodination which could be related to an abnormal T₄ uptake by the cell, particularly the hepatocyte. The low production of IGF-I could be related to a deficient GH receptor, as suggested by the decreased GH binding observed in the liver of dwarf birds. Both T₃ and IGF-I synthesis may share common pathways since thyroidectomy also decreases IGF-I level while a GH injection stimulates the T₄ to T₃ monodeiodination in the normal embryo but not in the dwarf. Further studies are needed on the GH receptor and the T₄ uptake in the hepatic cell to identify the common point where the dwarf gene could act. Ovulation rate and lipomobilisation are decreased in adult dwarfs but these findings are not yet easily related to the endocrinological changes observed during growth.

A complementary approach to compare the genotypes would be to study the DNA polymorphism using available molecular probes

sex-linked gene – dwarfism – chicken – endocrinology

Résumé — Synthèse des études physiologiques du nanisme lié au sexe chez la poule en vue de la recherche de l'effet primaire du gène. La récapitulation des résultats obtenus depuis dix ans sur les effets hormonaux et métaboliques du gène de nanisme lié au sexe chez la poule contribue à préciser son mode d'action sur la croissance et d'autres caractères.

Parmi les principaux facteurs endocriniens affectant la croissance, les hormones thyroïdiennes, T4 et T3, l'hormone de croissance, GH, et le facteur de croissance IGF-I ont été les plus étudiés chez les nains. Ces animaux montrent de faibles taux circulants de T3 et d'IGF-I malgré des taux normaux voire augmentés de T4 et de GH. La diminution de T3 s'explique par une moindre monodéiodination périphérique de T4, probablement reliée à une disponibilité insuffisante de T4 dans la cellule hépatique. La diminution d'IGF-I pourrait être rapprochée du déficit en récepteurs de GH, expérimentalement démontré chez les nains. Les synthèses de T3 et d'IGF-I pourraient partager des voies communes car la thyroïdectomie diminue aussi l'IGF-I circulante alors qu'une injection de GH stimule la monodéiodination de T4 en T3 chez l'embryon normal, mais pas chez le nain. Les études du récepteur à GH et des mécanismes d'entrée de T4 dans l'hépatocyte doivent être poursuivies pour préciser le point commun où pourrait intervenir le gène de nanisme. La diminution du taux d'ovulation et de la lipomobilisation observée chez l'adulte nain est encore mal expliquée. Une approche complémentaire pour comparer les génotypes serait d'étudier le polymorphisme de l'ADN avec les sondes moléculaires disponibles.

nanisme – gène lié au sexe – poulet – endocrinologie

Introduction

The sex-linked mutant gene (*dw*) causing dwarfism in the fowl (*Gallus domesticus*) was first described by Hutt (1959). It has been shown to occur in different populations around the world, as reviewed by Guillaume (1976). It has been generally assumed that dwarf mutations of different origin correspond to the same gene. A large number of genetic, zootechnical and physiological studies have already been carried out that have led to the commercial use of the mutation in the maternal lines of the broiler industry. However, the mode of action of the gene has not yet been precisely defined. Such knowledge would have both applied and fundamental consequences. Practical consequences would be to determine the effects of the gene on other traits such as ovulation rate.

The identification of the protein directly modified by the gene would provide a basis for the molecular cloning of the gene: a probe for the sex-linked dwarf mutation would be useful to compare the different mutants known in the world and eventually to prove their identity. Cloning the gene would also provide a tool to genetically manipulate growth in birds. From a more general point of view, dwarfism can also be used as a model to understand growth regulation.

This review will mainly deal with the results of the last 10 years that have been obtained on endocrinological and metabolic effects of the gene. The main factors involved in growth regulation will first be presented in the normal chicken, then the modifications observed in the growing dwarf chick will be described. A synthesis of these modifications may help to derive a working hypothesis on the probable primary effect of the gene. Some data obtained in the adult dwarf hen will then be briefly considered in connection with the physiological changes which are known in the dwarf chick. Finally, further experiments aimed at testing our hypothesis can be considered, in the field of physiology and perhaps also of molecular biology.

Hormones involved in growth in chicken

According to Scanes *et al.* (1984), growth hormone (GH) on the one hand and thyroid hormones (T3, T4) on the other hand are considered to be the main hormones required

for the normal growth of a chick. Growth factors are being increasingly studied, such as insulin-like growth factors I and II, IGF-I being also called somatomedin-C, epidermal growth factor (EGF), nerve growth factor (NGF) or vitamin D metabolites. Other important hormones such as insulin, glucocorticoids or steroids also play a role in growth regulation. The synthesis and release of GH by the pituitary is regulated by hypothalamic peptides, in a stimulating manner for growth hormone releasing factor (GRF) and thyrotrophin releasing hormone (TRH), in an inhibitory manner for somatostatin (SRIF). The roles of vaso-active intestinal peptide (VIP) and prolactin remain to be clarified so far as growth is concerned. These different hormones and growth factors have been presented in Figure 1, with their relationships and their sites of synthesis. Some comments appear particularly relevant in view of a study on the dwarf gene: the stimulating role of human GRF and TRH on GH secretion has been shown in several mammalian species and in chicken. Growth hormone elicits the synthesis of IGF-I in the rat liver (Schalch *et al.*, 1979; Mathews *et al.*, 1986), but other tissues are also able to produce IGF-I. In the chicken it has recently been demonstrated that an intravenous injection of GH was also able to increase the plasma concentration of IGF-I (Huybrechts *et al.*, 1988). The chicken thyroid gland mainly releases tetraiodothyronine (T4) in the bloodstream, together with only a small amount of triiodothyronine (T3) according to Lam *et al.* (1986). The major proportion of circulating T3 is produced by peripheral monodeiodination, which takes place in the liver and in other tissues. Both T3 and IGF-I exert a negative feed-back effect on GH secretion (Harvey, 1983; Buonomo *et al.*, 1987).

The changes introduced in this complex endocrinological system by the dwarf gene have been described at the levels of hormone production and tissue sensitivity, mainly by *in vivo* experiments with some *in vitro* studies.

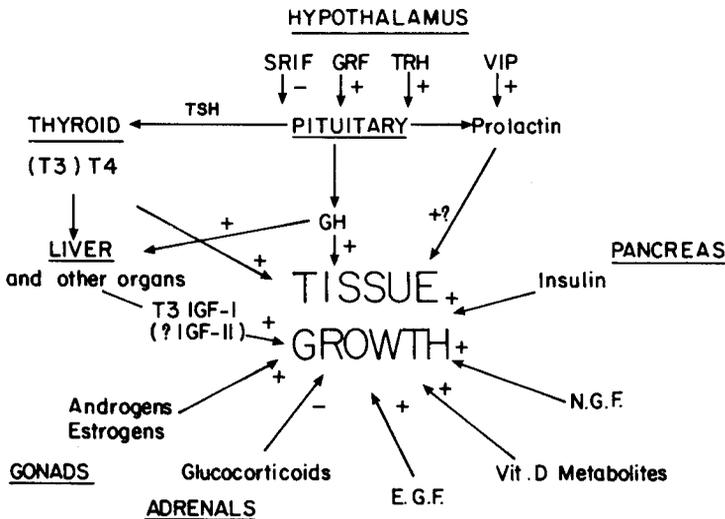


Fig. 1. Hormones involved in growth regulation in the chicken.

Effects of the sex-linked dwarf mutation during growth

State of knowledge in 1976

According to the review by Guillaume (1976), dwarf chicks exhibited hypothyroidism which could not be entirely held responsible for the dwarfism. No significant deficiency was found at the level of the thyroid gland. The saturation status of the thyroid hormone binding protein was used as a test to measure triiodothyronine plasma level and dwarfs showed higher *in vitro* uptake of T3 than the normal birds (Grandhi and Brown, 1975a). However, the authors suggested a failure to utilize thyroid hormones (Grandhi and Brown, 1975b) or a difference in the cellular transport of T3 or T4 (Grandhi *et al.*, 1975). Treatment with iodinated proteins (protamone) could not compensate for the final growth retardation of dwarfs. The avian growth hormone was not purified until 1974. No direct immunoassay of GH had been performed on dwarf birds. Somatomedins were known but could only be studied by biological assay: serum from dwarf chicks seemed to be able to enhance sulphation in chick cartilage as well as or even better than serum from normal chicks. The working hypothesis was thus a defect in tissue sensitivity to growth factors. This was in agreement with the observation that the difference between dwarf and normal birds remained after hypophysectomy.

These data have now been updated following the use of new technologies : radioimmunoassays for T4 and T3, GH (homologous), IGF-I (heterologous), assay for GH-receptors, *in vitro* techniques for cell physiology.

It should be noted that many studies compare a dwarf to a normal strain. However, a more accurate and reliable evaluation of the gene's effect can be obtained from the comparison between dwarf and normal full sibs, born from heterozygous sires and dwarf dams. A linkage with the feather color gene, silver, enables the distinction of dwarfs from normals to be made at the embryo stage (Huybrechts *et al.*, 1986). Two types of birds, broilers or laying strains, are also studied which may explain some of the differences between studies.

Recent endocrinological studies on dwarf mutation

Thyroid hormones

Descriptive study. Circulating levels of T3 and T4 did not differ between normal and dwarf embryos from 18 days of age to the time of hatching (Huybrechts *et al.*, 1986). However, an important difference arose after hatching: the T3 level exhibited a significant increase in the normal chick that was not seen to the same extent in the dwarf. Dwarfs showed lower levels of T3 from the age of 3 to 9 weeks, but slightly higher levels of T4 from the age of 1 to 7 weeks. Many authors now agree that sex-linked dwarf chicks have decreased circulating levels of T3 but normal or slightly increased levels of T4 (May and Marks, 1983; Scanes *et al.*, 1983; Stewart *et al.*, 1984; Hoshino *et al.*, 1986; Lauterio *et al.*, 1986). This has justified the study of peripheral monodeiodination in dwarfs.

Peripheral monodeiodination The pathways of thyroid hormone deiodination and their regulation are well described in the rat by *in vivo* and *in vitro* studies (Visser *et al.*, 1984). Different types of deiodination enzymes have been described: type I in the peripheral tis-

sues such as liver, kidney or muscle; type II in the brain and pituitary; type III in the brain and placenta. *In vitro* studies have not been developed to the same extent in the chicken. According to Fekkes *et al.* (1982), in the rat the same liver enzyme is responsible for the 5 and the 5' deiodination (5-D and 5'-D) leading respectively to reverse-T3 (rT3), inactive, or T3, active. Circulating level of rT3 was found to be similar and very low in normal and dwarf chicks, suggesting a rather normal 5-D in dwarfs (Hoshino *et al.*, 1986). The regulation of the 5'- or 5- site of deiodination is not fully understood, but could be related to intracellular pH (Visser *et al.*, 1979). The regeneration of the enzyme in its active form requires sulfhydryl groups (Visser *et al.*, 1976), which were shown to be normally present in the liver of dwarf birds (Decuypere, unpublished data). The deiodination activity is influenced by feeding status and temperature: fasting decreases it and refeeding has a stimulating effect on 5'-D in the chicken (Decuypere and Kühn, 1984). This refeeding effect could be related to insulin rather than to glucose, according to an *in vitro* study in the rat (Sato and Robbins, 1981). Cold also increases the deiodination activity (Rudas and Pethes, 1984, 1986).

Ovine prolactin, GH and also TRH were able to stimulate peripheral monodeiodination in the chick embryo and the adult chicken but not in the growing chick (Kühn *et al.*, 1983, 1988). Indeed, the sharp increase in T3 following the perforation of the inner shell membrane just before hatching is due to a higher conversion rate of T4 to T3 (Decuypere *et al.*, 1982). This increase in T3 could be induced as early as the age of 18 days by an injection of ovine prolactin or growth hormone, but such a stimulation could not be observed in dwarf embryos (Kühn *et al.*, 1986). This lack of T3 response in dwarfs was explained by a malfunction at the level of the peripheral monodeiodination. Similarly, a lower *in vitro* activity of the liver 5'-D was found in chicks of a dwarf Leghorn strain compared to a normal Leghorn strain (Scanes *et al.*, 1983). However, this could not be confirmed in comparing dwarf and normal sibs within a strain of brown-egg layers at 4, 8 and 12 weeks of age (Decuypere *et al.*, 1986). The *in vitro* assay was performed on the supernatant fraction obtained after centrifugation of homogenized liver tissue, and in this system the enzyme was shown to be present and to work normally. However, its activity appeared much reduced in its cellular environment (*i.e.*, *in vivo*). This could suggest a defect in the cellular environment of the enzyme and may in particular raise the question of the availability of T4 as a substrate to the enzyme. The T4 uptake into hepatic cell has been shown to depend on both a passive diffusion process and an active mechanism requiring ATP. Furthermore, in rats, the specific hepatic receptor sites for T4 may be different from the sites for T3 (Gharbi and Torresani, 1979; Krenning *et al.*, 1978, 1981). A defect at this level would be in agreement with a normal *in vitro* but reduced *in vivo* deiodination, as found in dwarfs. It is interesting to note that the hypothyroidism of dwarfs is not related to thyroid, but to the peripheral monodeiodination activity that was not studied until recently in relation to fowl dwarfism.

Stimulation experiments. The T4 release could be stimulated by TRH both in normal and dwarf chicks, with a tendency to show a delayed increase of T4 in dwarfs (Michels *et al.*, 1986; Hoshino *et al.*, 1986). Depending on age, TRH had no effect on T3 level in young chicks and elicited a slight increase of T3 at 11 weeks of age in normal birds only, again suggesting a lower ability of dwarfs to convert T4 into T3.

Supplementation experiments. Dietary T4 did not significantly change the growth curve of dwarf chicks from 1 to 9 weeks of age; however, a positive effect on body weight could be observed at 4 weeks of age in one experiment (Marsh *et al.*, 1984a). A high dose (10

ppm) significantly depressed growth (Leung *et al.*, 1984a). The T4 treatment slightly increased the T3 plasma level of dwarfs, but to a much lesser extent than the stimulation found in normals. A T3 dietary treatment could stimulate growth of dwarf chicks at a dose of 0.1 or 0.3 ppm in some but not in all studies (Leung *et al.*, 1984; Marsh *et al.*, 1984b; Bowen *et al.*, 1987; Tixier-Boichard *et al.*, submitted for publication). A genotype x treatment interaction was observed when normal chicks became hyperthyroid and had a reduced growth rate, while growth of dwarf chicks was improved or not affected. Higher doses (1 – 10 ppm) had a negative effect due to an induced hyperthyroid status in dwarfs (Leung *et al.*, 1984a; Scanes *et al.*, 1986; Tixier *et al.*, 1986). However, consistent metabolic effects were observed, suggesting a normal sensitivity of dwarf tissues to T3, such as an increased rectal temperature, a deterioration of feed efficiency, a decrease in abdominal fat content, a decrease in GH level as a negative feedback effect (Leung *et al.*, 1984a; Tixier *et al.*, 1986). This last point justified the simultaneous administration of GH in some experiments of T3 or T4 supplementation (Marsh *et al.*, 1984a; Bowen *et al.*, 1987). Together with T3, GH succeeded in stimulating growth of dwarf chicks, though treated chicks did not fully reach the body weight of the control normal chicks.

Growth hormone and insulin-like growth factor I

Descriptive studies. The plasma levels of GH and IGF-I did not differ between genotypes at the end of the embryonic life (Huybrechts *et al.*, 1987) as had already been observed in thyroid hormones. Indeed, no difference in body weight appears at hatching between genotypes. Dwarf chicks showed a normal or even higher circulating level of GH from the age of 3 to 9 weeks. The literature data show either a similar or an increased GH plasma level in dwarfs as compared to normals (Hoshino *et al.*, 1982; Scanes *et al.*, 1983; Stewart *et al.*, 1984; Lauterio *et al.*, 1986; Bowen *et al.*, 1987). However, IGF-I plasma level was always found to be reduced by about 50% (Hoshino *et al.*, 1982; Huybrechts *et al.*, 1985a; Bowen *et al.*, 1987). These results clearly show a deficient synthesis of IGF-I in dwarf birds, which constitutes new information regarding the mutation. The growth hormone of dwarf chicks is immunoreactive, although its bioactivity has not yet been proved, but electrophoretic studies suggested a good similarity between the circulating growth hormone found in dwarf and that found in normal birds (Hoshino and Suzuki, 1982).

Stimulation studies. The increase in GH plasma level after injection of hGRF or TRH was of greater magnitude and lasted longer in dwarf than in normal birds (Hoshino *et al.*, 1984; Huybrechts *et al.*, 1985b). TRH administration could also increase the GH plasma level in adult dwarf hens, but had no effect in normal hens (Harvey *et al.*, 1984; Scanes *et al.*, 1986). These results suggested that dwarfs showed a higher sensitivity to growth hormone releasing factors or a greater pool of GH because of their lower T3 circulating level, which normally exerts an inhibitory action on GH synthesis and release in birds. Surgical or chemical thyroidectomy has been shown to promote TRH-induced GH secretion in adult birds (Harvey *et al.*, 1988). Furthermore, the dietary administration of T3 to dwarf birds could decrease a TRH-induced GH secretion (Scanes *et al.*, 1986; Tixier-Boichard *et al.*, submitted for publication). The slower decrease of GH circulating level could also indicate a slower rate of peripheral utilization of the hormone.

The stimulation of IGF-I synthesis by GH appeared to take place in dwarfs, but the relative increase in IGF-I plasma level was much smaller than in normal birds (Huybrechts *et al.*, 1988).

GH receptors. The low sensitivity to GH, as recently described by Huybrechts *et al.* (1988), combined with the deficiency of basal IGF-I production in dwarfs, in spite of high levels of circulating GH, could suggest a lack of response of the hepatic cell to GH. Indeed, hepatic binding of avian GH has been shown to be markedly reduced or almost absent in dwarf birds of 2 different strains (F.C. Leung *et al.*, 1987). These results also suggested a role for the genetic background since no GH-binding at all was noticed in dwarfs from a laying strain (Leghorn), while a few dwarf birds from a broiler strain exhibited a low binding level. The authors found a higher correlation between growth rate and hepatic GH binding than between growth rate and GH plasma level. However, the possibility of a down-regulation of the GH receptors by the hormone itself cannot yet be ruled out in dwarfs and needs further investigation.

IGF-I receptors. The receptors for IGF-I have not yet been directly studied in dwarf birds. *In vitro* studies did not show any systematic difference in the basal level of cartilage sulfation activity or DNA synthesis between dwarfs and normals as shown by a between-strain comparison at 7 weeks of age (Hoshino *et al.*, 1982) and by a within-strain comparison from 17 days of incubation until 5 weeks of age (Charrier, unpublished results; Figure 2). The addition of normal chicken serum at the concentrations of 2.5 and 5% in the incubation medium of cartilage tended to stimulate the cartilage activity in dwarfs and in normals, but only in a few cases so that no significant differences could be established between genotypes (Hoshino *et al.*, 1982; Figure 3). However, the addition of 20% of normal chicken serum to the incubation medium could stimulate the sulfation activity of cartilage with a significantly higher response in dwarfs at 3, 7 and 13 weeks of age but not at 5 weeks of age (Charrier, unpublished data; Figure 4). DNA synthesis could

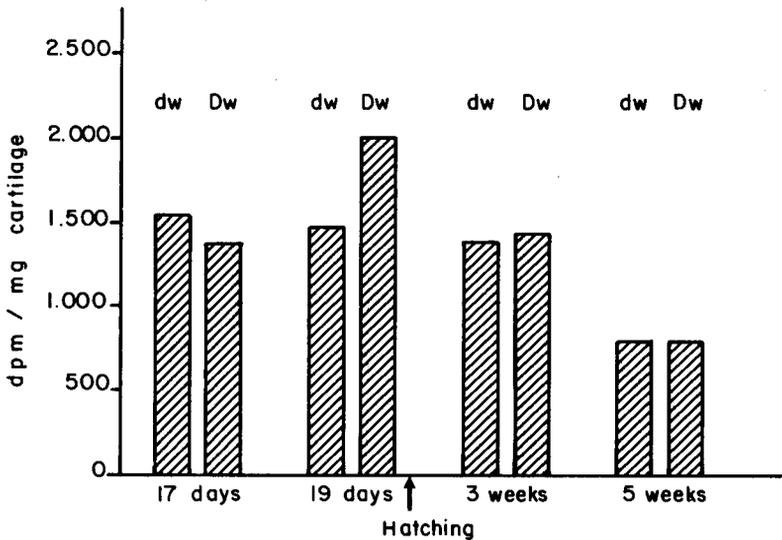


Fig. 2. A within-strain comparison of the spontaneous incorporation of ^{35}S -sulfate into sternal cartilage in sex-linked dwarf (*dw*) and normal (*Dw*) birds from 17 days of incubation until 5 weeks of age.

only be enhanced by the addition of 20% normal serum at 7 and 13 weeks, with no significant differences between genotypes.

These results showed a normal activity of cartilage in dwarfs, with a somewhat higher sensitivity to serum growth factors, but they should be confirmed on a larger sample of animals, with the use of defined growth factors in the culture medium. It must also be emphasized that these data were obtained from laying strains and that the situation might differ in heavy strains.

Corticosteroids

Dwarf birds showed a lower circulating level of corticosterone from 1 to 6 weeks of age and no further difference at 12 weeks of age. Furthermore, the plasma corticosterone response to an acute heat stress at 12 weeks of age was lower in dwarfs than in normals. *In vitro* studies also suggested a decreased cellular sensitivity to ACTH of the adrenal gland in dwarfs as compared to a normal strain (Carsia and Weber, 1986). These results are not particularly related to the decreased growth in dwarfs, but the behavioral aspects and response to stress are interesting to consider. However, age should be taken into account since adult dwarf hens were shown to have higher corticosterone plasma levels, in a fed state as well as during starvation (Rombauts *et al.*, 1983).

Insulin

Dwarf birds showed rather lower levels of insulin after refeeding or a glucose load (Simon, 1983). However, glucose plasma level was not different from the control after refeeding. A glucose load induced a similar increase in glucose plasma level in both

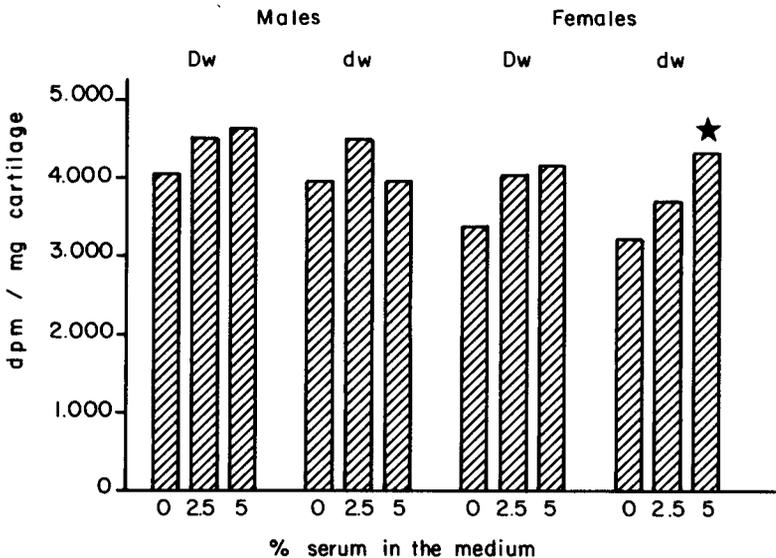


Fig. 3. Stimulation of the incorporation of ^{35}S -sulfate into sternal cartilage by addition of normal serum in the incubation medium: comparison between a dwarf and a normal Leghorn strain at 7 weeks of age. *: significantly different ($P < 0.05$) from the within sex-genotype control.

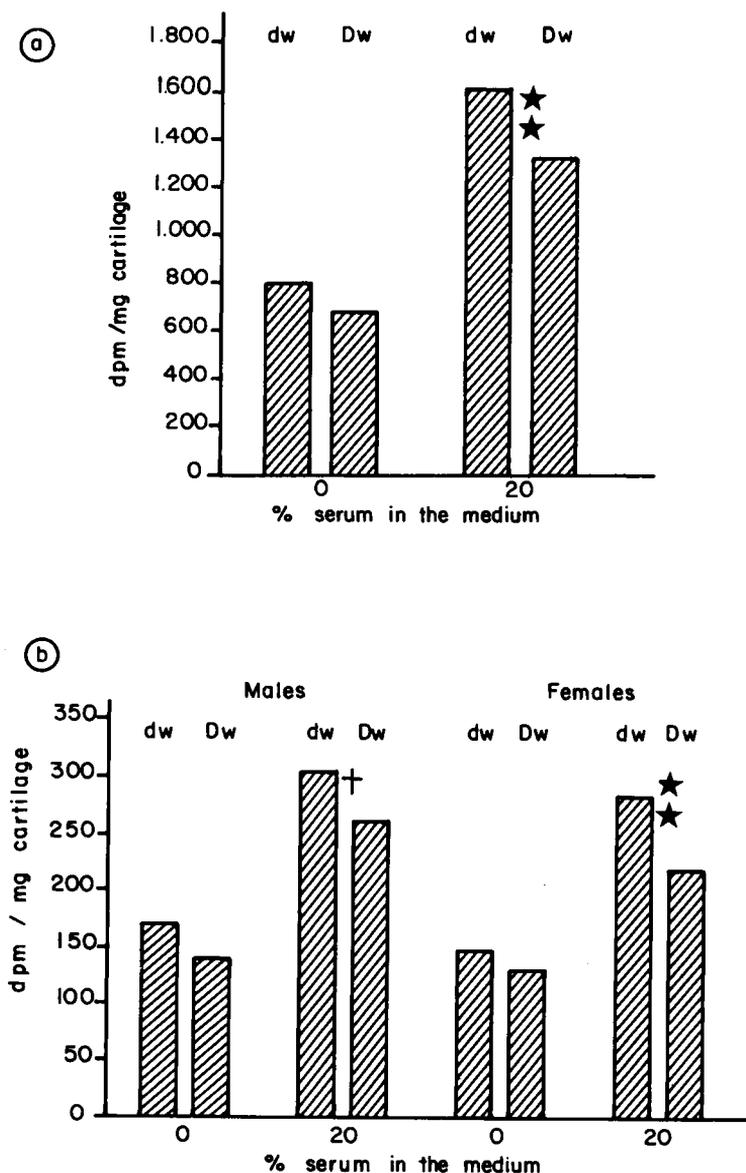


Fig. 4. Stimulation of the incorporation of ^{35}S -sulfate into sternal cartilage by addition of normal serum in the incubation medium: a within-strain comparison between sex-linked dwarf (*dw*) and normal (*Dw*) chicks. **a:** at 3 weeks of age in a brown-egg type strain; **b:** at 7 weeks of age in a Leghorn strain; significantly different at $P < 0.10$ (+) (or $P < 0.01$ (**)) from the other genotype in the same treatment group.

genotypes, but it lasted slightly longer in dwarfs before returning to the same basal level, 90 minutes after the initial load. These results suggested an increased sensitivity to insulin action that could be related to an inefficient growth hormone.

Summary of the effects of the dwarf gene during growth

The modifications induced by the sex-linked dwarf gene during growth are presented in Table 1. The Mendelian inheritance of dwarfism suggests the search for a single abnormality which could explain the different effects observed. On the one hand, dwarfs have a reduced synthesis of T3 in the presence of an increased T4 level, which could be related to lower *in vitro* monodeiodination activity, in spite of the presence of the enzyme; on the other hand, the synthesis of IGF-I is depressed and the GH hepatic binding activity is reduced or almost absent, in the presence of a normal or even increased GH circulating level. The primary effect of the gene may thus be at an interaction point between the GH and IGF-I axis and the thyroid hormones.

In normal birds, chemical or surgical thyroidectomy decreased the circulating level of IGF-I but increased the GH level (Decuypere *et al.*, 1987). Chemical thyroidectomy was achieved by the administration of methimazole (MMI); in that case growth of normal chicks was greatly impaired because not only thyroid hormones but also IGF-I levels were decreased. This could suggest that a normal thyroid function is required for a proper synthesis of IGF-I. Treatment of dwarf birds by T3 had a strong negative effect on GH level but affected IGF-I level to a much lesser extent (Tixier *et al.*, 1986). These data should be confirmed. If the T3 deficiency could be held responsible for the low synthesis of IGF-I, the primary effect of the gene could take place at the level of the T4 uptake by the hepatic cell, since the monodeiodination enzyme was shown to be present in dwarfs. This hypothesis would require the study of mechanisms for transport of T4 into the cell,

Table 1. Summary of the endocrinological changes due to the sex-linked dwarf gene during growth.

<i>Hormone</i>	<i>Basal level</i>	<i>Stimulating factor (s)</i>	<i>Effect</i>
T3	decreased	TRH	decreased due to low deiodination
T4	increased	TRH	almost normal
GH	increased	TRH, GRF	increased
IGF-I	decreased	GH	decreased
Corticosterone	decreased	stress	decreased
Insulin	decreased	glucose	decreased

<i>Receptor</i>	<i>Quantity</i>	<i>Affinity</i>
GH	decreased or absent	decreased ?
T4	decreased ?	decreased ?
IGF-I	unchanged ?	

either passive or active (Krenning *et al.*, 1982). T4 administration to dwarf chicks had some physiological effects on body weight, on T3 and GH plasma levels, suggesting that these birds were not resistant to the hormone. The T4 metabolism abnormality in dwarfs does not seem to be an all-or-none trait. A normal passive transport may explain the effects observed with T4 administration, but the low level of T4 to T3 conversion and its lack of response to an injection of GH or prolactin in the embryo may imply a defect in a regulated active transport. The absence of the receptor cannot be ruled out, but the defect could be due to a change in receptor affinity, or to a modified regulation process at a level which has yet to be determined.

Reciprocally, GH has been shown to stimulate the T4 to T3 conversion in the normal embryo, with no effect in the dwarf. In the chick, this GH effect is no longer seen, but the conversion of T4 into T3 occurs at a much higher rate than in the embryo. Is it possible that a hypothetical positive effect of GH on the T4 to T3 deiodination might no longer be enhanced by exogenous hormone at that age? Considering that growth hormone may be required for a normal peripheral deiodination of T4 into T3, a GH receptor defect could then be seen as a sufficient cause of both deficiencies in IGF-I and T3 production. The injection of GH into dwarfs of a brown-egg type layer strain induced only a small increase in the circulating level of IGF-I (Huybrechts *et al.*, 1988). This small effect alone could be seen as a sufficient reason to exclude the total absence of the receptor in dwarfs. However, the results obtained in the Leghorn strain by F.C. Leung *et al.* (1987) suggested that the GH receptor could be totally lacking in dwarfs or have lost any binding affinity for the hormone, but the results were not as clear-cut in the broiler strain. Further experiments are required to test the hypothesis of an abnormal GH receptor in dwarfs. An interesting stage to study would be the end of the incubation period where the normal increase of T3 production does not take place to the same extent in dwarfs and cannot be stimulated by GH as in normals.

The target tissues such as cartilage or muscle have not been extensively studied in dwarfs. Normal cartilage may be able to produce its own growth factors, in an autocrine manner (Burch *et al.*, 1986), so that it would not be entirely dependent on the circulating IGF-I. However, the circulating IGF-I can still play a role, as shown by the effects of exogenous IGF-I on growth of miniature poodles (Guler *et al.*, 1987) and of hypophysectomized rats (Schoenle *et al.*, 1982). In addition to *in vitro* studies, the simultaneous compensation for both T3 and IGF-I deficiencies in dwarfs would permit an *in vivo* evaluation of the growth potential of peripheral tissues.

Relationships between the effects of the gene during growth and its effects in the laying hen will be considered below.

Effects of the gene during the laying period

Descriptive studies

The sex-linked dwarf gene reduces vitellogenesis and ovulation rate (Guillaume, 1976; Leenstra *et al.*, 1986). However few results have been obtained on the steroid hormones of the dwarf laying hen. Recent studies tend to show a significant effect of the mutation

on the basal plasma levels of 17β -estradiol, which would be increased, and of progesterone, which would be decreased (Tixier-Boichard and Rombauts, 1988). Consequently, the ratio of estradiol to progesterone would be higher in dwarfs. This result was most noticeable after the laying peak whereas no trend appeared earlier. The relationship of this change in the circulating levels of sex steroids to the other endocrinological effects of the mutation remains to be elucidated. It could also be a direct consequence of the reduced number of fast-growing ovarian follicles in the dwarf hen. Furthermore, no significant change was observed in the luteinizing hormone plasma level around the onset of laying, which could indicate a peripheral defect; but the ovarian sensitivity to pituitary factors has not been studied in the dwarf hen.

So far as vitellogenesis is concerned, the dwarf Leghorn hen appeared to be more dependent on the diet for the lipid composition of the yolk (Demarne *et al.*, 1984; Burghelle-Mayeur, 1988). The *in vitro* lipolytic and lipogenic activities of the adipose tissue were significantly lower in dwarf Leghorn hens while hepatic lipogenic activity was not changed (Burghelle-Mayeur, 1988). Adult dwarf hens have much less abdominal fat than their normal sisters (Merat and Ricard, 1974), but the opposite is observed at a young age where the dwarf chicks are fatter (Stewart *et al.*, 1984). The effect of the dwarf gene on lipid metabolism during growth is not yet clearly explained: lipogenesis was found to be decreased in a dwarf line at 4 weeks of age (Rosebrough *et al.*, 1986), but contradictory results were obtained depending on the genetic background where the gene was introduced (Calabotta *et al.*, 1983). Results seem to be more consistent regarding lipolytic activity which appeared to be decreased in dwarf chicks (Guillaume, 1976; Calabotta *et al.*, 1983). The balance between lipogenesis and lipolysis may differ between the growing and the laying periods, but a constant feature in dwarfs seems to be a reduced ability to mobilize their lipid deposits. This decreased lipolytic activity may be related on the one hand to the decreased T3 level, since T3 is lipolytic in the chicken (Decuyper *et al.*, 1987), and on the other hand to a deficient growth hormone or to a defect of the GH receptor since growth hormone has been shown to be involved in the lipid metabolism of the chicken, with either a lipolytic activity *in vivo* (Campbell and Scanes, 1985) and *in vitro* (Hall *et al.*, 1987), or an antilipolytic action *in vitro* consisting of a short-term inhibition of the glucagon-induced lipolysis in the adipose tissue (Campbell and Scanes, 1987). The role of growth hormone in the regulation of lipolysis could thus be interesting to study in the dwarf hen.

Consequences of the hormonal deficiencies associated with dwarfism during the laying period

Lower T3 production

T4 plasma levels were increased during the laying period in dwarf Leghorn hens as compared to normal hens, while the circulating levels of T3 were rather decreased, this pattern being very close to the situation described at an early age (Decuyper, unpublished data). In a preliminary experiment, where a small sample of dwarf hens received 0.1 ppm T3 in the diet from 18 to 22 weeks of age, the plasma level of T3 was slightly increased while the T4 level was decreased, but no significant effect could be noticed on laying traits (Tixier-Boichard, unpublished results). An inverse relationship is usually described between the gonadal and the thyroid function, particularly at the onset of broo-

diness (Sharp and Klandorf, 1985). Thyroid hormones seem to have an inhibitory action on the secretion of luteinizing hormone in the fowl (Harvey *et al.*, 1983). They also appeared to have an inhibitory effect on the estrogen-induced vitellogenin synthesis in the chick embryo liver (Elbrecht and Lazier, 1985). However, a minimal level of thyroid hormones may be necessary for a chicken to exhibit a normal response to an estrogen challenge, as shown in the obese strain of *White Leghorn* showing inherited hypothyroidism (Schjeide *et al.*, 1986). Sex-linked dwarf cockerels have a T3 deficiency, but appeared to be as sensitive as their normal half-brothers in response to an injection of 17 β -estradiol (5 mg/kg) at 7 weeks of age: the plasma level of triglycerides reached a maximum 28 h after the injection, with a mean (\pm SEM) of 5.88 ± 0.94 g/l in dwarfs as compared to 1.95 ± 0.72 g/l in untreated dwarfs and a mean of 7.19 ± 0.28 g/l in normals as compared to 1.96 ± 0.58 g/l in untreated normals (Tixier-Boichard, unpublished results).

The changes in thyroid metabolism due to the dwarf gene are not easily related to the effects of the gene on laying traits, indicating that the relationships between thyroid hormones and steroid hormones during reproduction should be further studied in the dwarf hen.

Lower IGF-I production

A possible role of IGF-I in the reproductive function has not yet been investigated in the chicken. In mammals, some studies suggest an IGF-I involvement in the reproductive function, as for instance the synergism with Follicle Stimulating Hormone to induce receptors for Luteinizing Hormone in cultured rat granulosa cells (Adashi *et al.*, 1985). The plasma level of IGF-I has been found to be reduced in dwarf pullets aged 15 and 18 weeks (Huybrechts *et al.*, 1987) but there are no data at an older age.

Until now, the effects of the sex-linked dwarf gene on reproduction and mainly on ovulation rate appear complex and may involve several regulation pathways that cannot be easily related to the marked modifications observed at a young age.

Conclusions and further approaches

The studies of the sex-linked dwarf mutation in growing chicks tend to favour the idea that the primary effect of the gene is connected with the receptor protein for growth hormone, from direct experimental evidence, or with the receptor for T4, from indirect experimental evidence.

Further physiological investigations are needed, including *in vitro* studies of T4 uptake and GH binding in the liver as well as *in vivo* studies of GH and IGF-I effects in the dwarf. From another point of view, molecular genetics can offer a new tool to test the hypothesis of an abnormal GH hormone or GH receptor in the dwarf. The gene for chicken GH has been cloned (Souza *et al.*, 1984) and could be used as a probe to detect a structural difference of the GH gene between dwarf and normal chicks, according to the method of restriction fragment length polymorphism. This difference should not be connected with the antigenic properties of the hormone, since it is normally recognized with the usual radioimmunoassays. Preliminary results obtained with the chicken GH probe were pre-

sented by Leung *et al.* (1984b) , showing no difference between the DNA from dwarf or normal birds. The rabbit gene for the growth hormone receptor has now been cloned (D.W. Leung *et al.*, 1987) and could provide an interesting tool to compare the dwarf to the normal genome. However, the mutation may be connected with only a part of the gene, the size of which is unknown. Since the entire probe may not be able to detect a small difference, a modified or an incomplete probe could then be useful. One can speculate on a mutation of that gene that could affect the sequence involved in the binding of the hormone to its receptor. If this sequence could be identified, it would help in establishing a "specialized" probe to be used in the dwarfs suspected of a GH-binding defect. These considerations are speculative, but are aimed at showing that both physiology and molecular genetics could now play a part in elucidating the nature of the dwarf mutation.

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