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Estimation of Thyroid Gland Activity in the Snell Dwarf Mouse by Ultrastructural Observation of the Thyroid Gland, Measurement of Plasma Thyroxine Concentration and Thyroid Hormone Binding Capacity

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Abstract

An ultrastructural study of the thyroid gland of the Snell dwarf mouse showed cellular activity to be very low. Follicle cell diameters were significantly lower than in controls whilst the nucleo-cytoplasmic ratio was significantly higher.

The observed cellular activity of the thyroid cells was associated with the circulating levels of thyroxine which were found to be significantly lower than in controls. Measurement of the free thyroxine index showed very little free hormone available for tissue uptake. No differences in thyroid function due to age or sex in the dwarf mice were seen.

Possible endocrine imbalances contributing to the low thyroid activity in the Snell dwarf mouse are discussed.

Introduction

In 1929 Snell reported dwarfism in mice and demonstrated that the mutation responsible was a recessive Mendelian character. Growth retardation and sterility has subsequently been demonstrated in the dwarf mouse (Smith and Macdowell 1931). The primary cause of this dwarfism has for some time been attributed to an adeno-hypophyseal deficiency (Carsner and Rennels 1960). Recent work by Roti *et al.* (1978) indicates that this may be the case as the dwarf mouse does not respond to administration of thyrotrophin releasing hormone (TRH) with secretion of thyroid stimulating hormone (TSH). Pituitary basophils (thyrotrophs and gonadotrophs) have been found in the dwarf mouse (Ortman 1956; Peterson 1959) although the thyrotrophs were few in number (Bartke 1964). Acidophils (luteotrophs and somatotrophs) in the dwarf mouse pituitary have been found to be low in number compared with control animals (Bartke 1964). Growth hormone (GH) and prolactin (LTH), as measured by radioimmunoassay, have also been reported to be low (Sinha *et al.* 1975).

In a recent study of the ultrastructure of the dwarf mouse thyroid it was suggested that the origin of the thyroid anomaly in dwarf mice was due to a drastic diminution in cell division (Cordier *et al.* 1976). In this study we have examined the effect of the dwarf mutation on thyroid function. Accordingly, circulating concentrations of thyroxine (T_4) were measured by radioimmunoassay together with serum thyroid hormone binding as measured by radioactive thyroid hormone uptake *in vitro* in

both dwarf and phenotypically normal mice. These findings were then related to an ultrastructural study of their thyroid glands.

Materials and Methods

Origin of Experimental Animals

The Snell dwarf mouse strain (genetic symbol *dw/dw*) used at Macquarie University originated from breeding pairs obtained from a colony maintained by the Division of Animal Production, CSIRO, Blacktown, N.S.W. The colony originated from a heterozygote (+/*dw*) female which was imported by the Genetics Research Laboratory, Division of Animal Health, CSIRO, Ryde, N.S.W., from the Institute of Animal Genetics, Edinburgh, U.K., in 1952. Since the homozygous dwarf is sterile the dwarfs were bred from heterozygous pairs.

Housing

Animals were housed under identical conditions in groups of 10 with food and water provided *ad libitum* and subjected to a natural day–night regimen. Pregnant females were separated into individual cages and dwarfs removed from their normal litter mates at approximately 28–30 days of age. Food and water were put into the cages of the dwarfs as they had difficulty reaching it if put in the normal position on the cage lid.

Plasma Thyroxine and Thyroid Hormone Binding Assays

Animals of both sexes were killed by cervical dislocation, exsanguinated and plasma samples were frozen for assay. Because of the small size of the dwarfs, in some cases pooled plasma samples were used. A minimum of five separate samples from each group was collected. Two age groups were studied, namely 30 and 90 days, the former being not yet sexually mature and the latter being sexually mature.

Thyroxine was assayed by radioimmunoassay using the Gammacoat™ [^{125}I]T_4 radioimmunoassay kit supplied by Clinical Assays (Division of Travenol Laboratories Inc., Cambridge, Mass. U.S.A. 02139). An antibody (raised in rabbits to a thyroxine conjugate) which is bound to a polypropylene tube, thereby simplifying separation, is used in the assay. The T_4 antibody cross-reacts significantly only with D-thyroxine (95%) and L-triiodothyronine (4·8%). Assay sensitivity was 0·39 μg/100 ml. Intra-assay and interassay variation were each approximately 10%.

Radioactivity was estimated with a Nuclear Enterprises NE1600 16-place manual counter (Nuclear Enterprises Ltd, Edinburgh EH11 4EY, Scotland). Unknowns were read from a standard curve with a computer program using a linear transformation (logit B/B_0 *v.* log concentration) and linear interpolation.

Serum thyroid hormone binding was determined using the Triobead™ [^{125}I]T_3 uptake kit from Abbott Laboratories. Serum was incubated with [^{125}I]triiodothyronine in the presence of activated charcoal which was immobilized on a bead trapped in the assay tube. The liquid was then decanted and the radioactivity bound to the charcoal counted. Results were expressed as a percentage of the label bound to the charcoal. A reference sample included with the kit is used to adjust the binding results so that 39·6% binding of label is assigned an arbitrary value of 100%. As it is the label bound to the charcoal which is counted, there is an inverse relationship between the serum thyroid hormone binding result and the available binding sites on the thyroid binding serum proteins. Intra-assay and interassay variation were each approximately 5%.

Free thyroxine index (FTI) was calculated for each T_4 and thyroid hormone binding result using the formula

$$\text{FTI} = (T_4 \times \text{thyroid hormone binding}) \div 100.$$

Ultrastructure of the Thyroid Gland

The thyroids of five dwarfs and five control animals from each sex from the two age groups were rapidly removed and placed in 4% (v/v) gluteraldehyde in 0·1 M phosphate buffer, pH 7, for 2 h at 5°C. The tissues were then post-fixed in 2% (w/v) osmium tetroxide for 1·5 h, dehydrated and embedded in Araldite. Silver sections were stained by the triple stain method modified by Soloff (1973). The sections, which were mounted on copper grids coated with nitrocellulose and carbon, were treated with a 0·9% (w/v) $KMnO_4$ solution buffered at pH 6·5 with phosphates for 2 min.

They were then treated with uranyl acetate saturated in 50% (v/v) ethanol for 3 min followed by 0·2% (w/v) lead citrate solution for 3 min. This method of staining increased electron density of the membrane systems.

The sections were examined using a Jeol X100 electron microscope. Because of the small size of the dwarf mice their thyroids were removed still attached to their surrounding tissue and fixed.

A Perspex plate that was gridded in a horizontal, vertical and two 45° tangents was used to measure the diameters of the nuclei and cells of thyroid follicle cells on photomicrographs which were taken at a magnification of 5000 times. The mean of those four measurements was then taken as a representation of cell or nuclear diameters and converted into micrometres.

Results

Plasma Thyroxine and Thyroid Hormone Binding

No significant changes due to age or sex were seen in thyroxine (T_4) concentrations or serum thyroid hormone binding in the dwarf groups. However, in the female controls the thyroid hormone binding in the 30-day group was significantly higher ($P<0·05$) than in the 90-day group. No other significant differences due to age or sex were found in the control animals (Table 1).

Table 1. Mean values ± s.e. for total thyroxine (T_4), serum thyroid hormone binding (THB) and free thyroxine index (FTI) in Snell dwarf mice and phenotypically normal mice with regard to age and sex

Thyroxine levels expressed as μg/100 ml plasma and thyroid hormone binding as a percentage of hormone bound. FTI is defined in the text. Number of mice in each experimental group given in parentheses

Assay	Age (days)	Sex	Control mice		Dwarf mice	
T_4	30	M	3·63±0·53	(5)	0·34±0·08	(5)
THB	30	M	150±7	(5)	116±11	(5)
FTI	30	M	5·4±0·9	(5)	0·4±0·1	(5)
T_4	90	M	3·34±0·62	(7)	0·54±0·12	(5)
THB	90	M	155±4	(7)	94±2	(5)
FTI	90	M	5·2±0·9	(7)	0·5±0·1	(5)
T_4	30	F	4·45±0·44	(6)	0·42±0·08	(5)
THB	30	F	173±7	(6)	96±14	(5)
FTI	30	F	7·7±1·0	(6)	0·4±0·1	(5)
T_4	90	F	4·12±0·27	(10)	0·72±0·19	(5)
THB	90	F	156±2	(10)	97±2	(5)
FTI	90	F	6·4±0·4	(10)	0·7±0·2	(5)

Plasma thyroxine concentrations were found to be significantly lower ($P<0·001$) in dwarf mice compared with control animals. The mean grouped male and female control (±s.e.) thyroxine concentration was found to be 3·92±0·23 μg/100 ml plasma whilst that for the dwarfs was found to be 0·51±0·07 μg/100 ml plasma.

Thyroid hormone binding was also seen to be significantly lower ($P<0·001$) in the dwarf mice compared with that in controls signifying fewer binding sites in the dwarf. A mean control value (±s.e.) of 159±2·6% bound was found compared with the dwarf value of 101±4·5% bound. Therefore the FTI was also found to be significantly lower ($P<0·001$) in dwarfs than in control mice. The mean FTI value for controls (±s.e.) was 6·2±0·4 compared with the dwarf value of 0·5±0·07 (Table 1).

Ultrastructure of the Thyroid Gland

No significant changes due to age or sex were seen in the ultrastructure of the thyroid follicle cells in either the control or dwarf groups.

It was observed that the dwarf thyroid gland had large areas of adipose tissue which were absent in the controls. Cytoplasm was sparse in the dwarf mouse thyroid cells with the rough endoplasmic reticulum not well developed. The mitochondria appeared to be less active in the dwarfs and there were also fewer Golgi complexes visible. These changes in the fine structure, however, were not quantified but scored subjectively on a random basis. The overall follicle size was greater and there were a larger number of follicle cells in the control animals compared with the dwarf (see Figs 1–4). No significant differences in nuclear or cell sizes of thyroid follicle cells with regard to age or sex in the control mice were found. Therefore the results obtained for the control mice at different ages and sexes were pooled and compared with those of the pooled dwarf groups. Results are tabulated in Table 2. Cell

Table 2. Diameters of thyroid follicle cells and nuclei (±s.e.) and ratio of nucleus to cell diameter (±s.e.) in Snell dwarf mice and phenotypically normal controls

n, Number of cells measured

Age (days)	Sex	*n*	Diameter (μm) Nucleus	Cell	Ratio of nucleus to cell diameter
			Control mice		
30	M	9	5·54±0·25	9·04±0·47	0·62±0·02
30	F	10	5·18±0·32	8·79±0·31	0·59±0·03
90	M	7	5·08±0·19	8·00±0·34	0·64±0·03
90	F	10	5·13±0·21	8·74±0·61	0·60±0·03
			Dwarf mice		
30	M	26	4·72±0·14	6·25±0·21	0·77±0·02
30	F	20	4·88±0·18	6·30±0·19	0·78±0·02
90	M	19	4·62±0·13	6·20±0·20	0·76±0·01
90	F	18	4·47±0·16	5·69±0·18	0·79±0·01

measurements demonstrated that in the female dwarf there was a significant difference in cell and nuclear sizes of the thyroid follicle cells between the two age groups, the 90-day age group sizes being significantly ($P<0·05$) lower. However, no significant difference was found in nuclear or cell sizes of thyroid follicles between the two age groups of male dwarfs. Cell diameters of the dwarfs were found to be significantly ($P<0·001$) smaller than in the control animals in all age groups where mean values (±s.e.) for controls were found to be 8·68±0·46 μm compared with 6·12±0·20 μm for dwarfs. With the exception of the 30-day females the nuclear diameters of the dwarfs were found to be significantly ($P<0·05$) smaller than in the control animals. The mean value (±s.e.) for controls was found to be 5·23±0·25 μm compared with that of 4·69±0·15 μm for the dwarfs. Furthermore, the nucleus to cell diameter ratio for the follicle cells was found to be significantly ($P<0·005$) larger in the dwarfs than in the control animals in both sexes and age groups. The mean nuclear cell diameter ratio (±s.e.) of controls was 0·61±0·01 compared with a value of 0·77±0·01 in the dwarf cells.

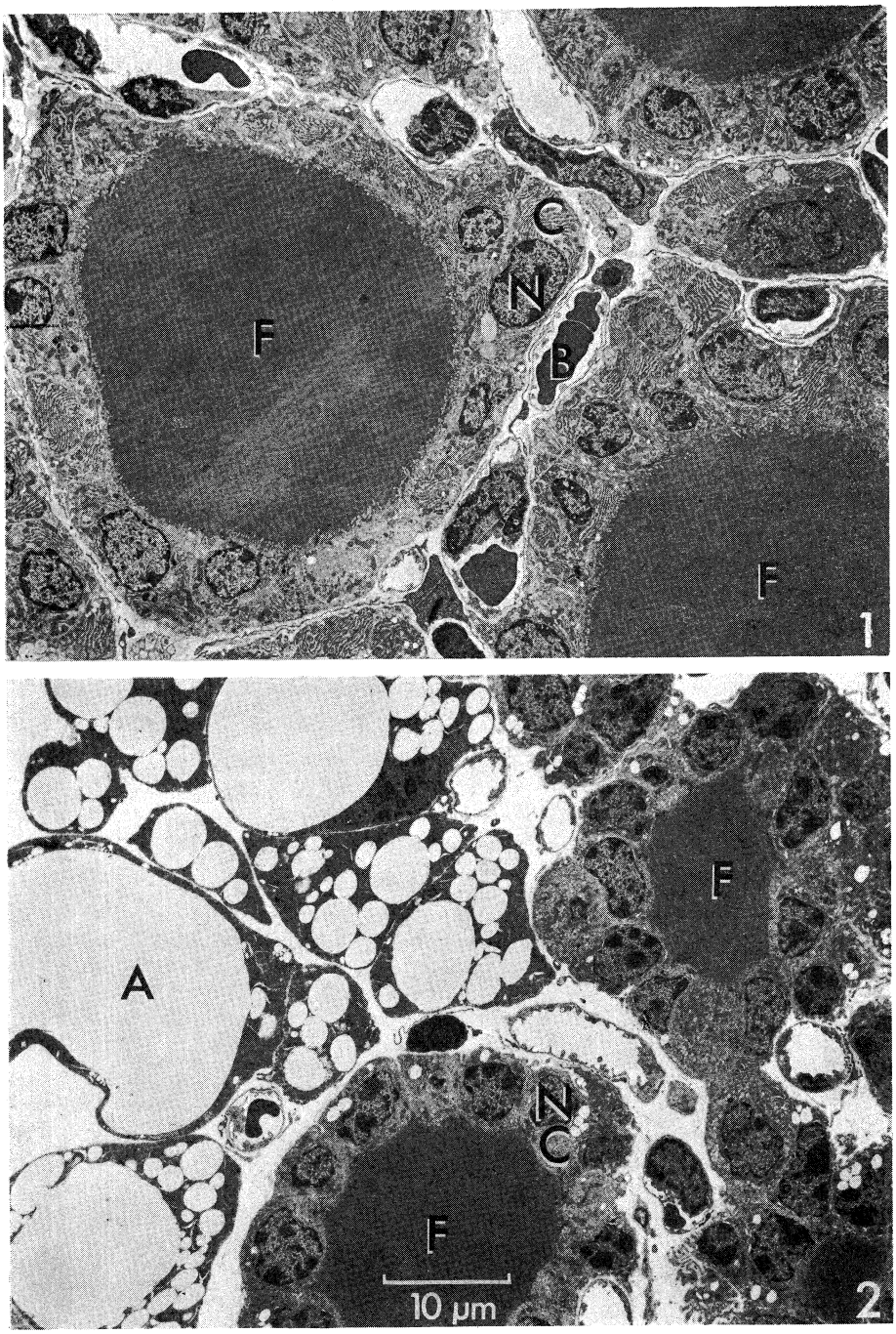
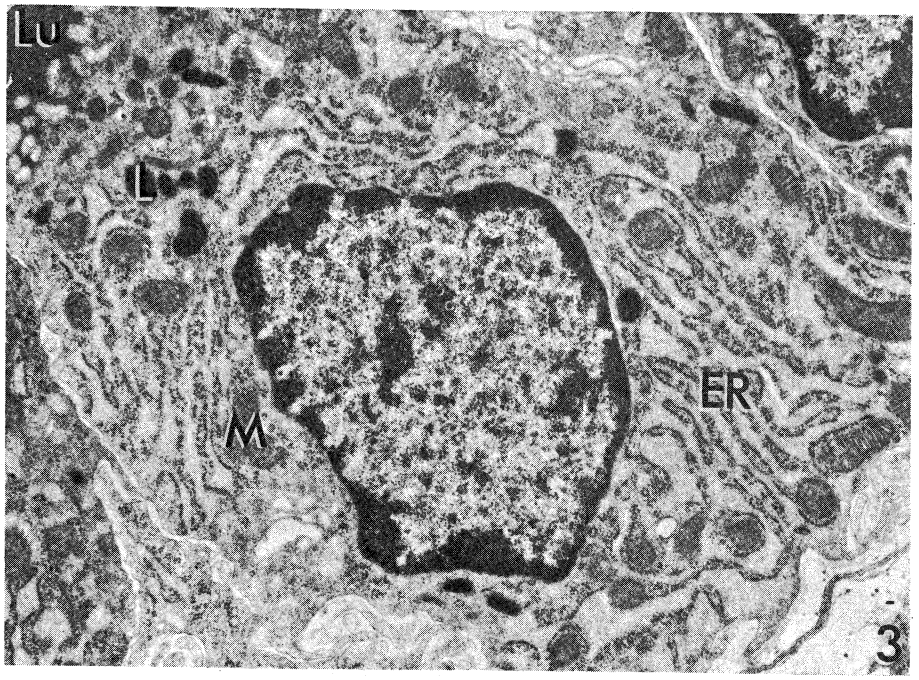


Fig. 1. Typical thyroid follicles (*F*) from a control mouse. The active follicle cells contain a large amount of cytoplasm (*C*) relative to the size of the nucleus (*N*). *B*, blood vessel containing a red blood cell.

Fig. 2. Typical thyroid follicles (*F*) from a dwarf mouse. Overall follicle size compared with that of the control mouse is smaller. The follicle cells of the dwarf thyroid appear to be inactive with a small amount of cytoplasm (*C*) in relation to the size of the nucleus (*N*). Note the large accumulation of adipose tissue (*A*).



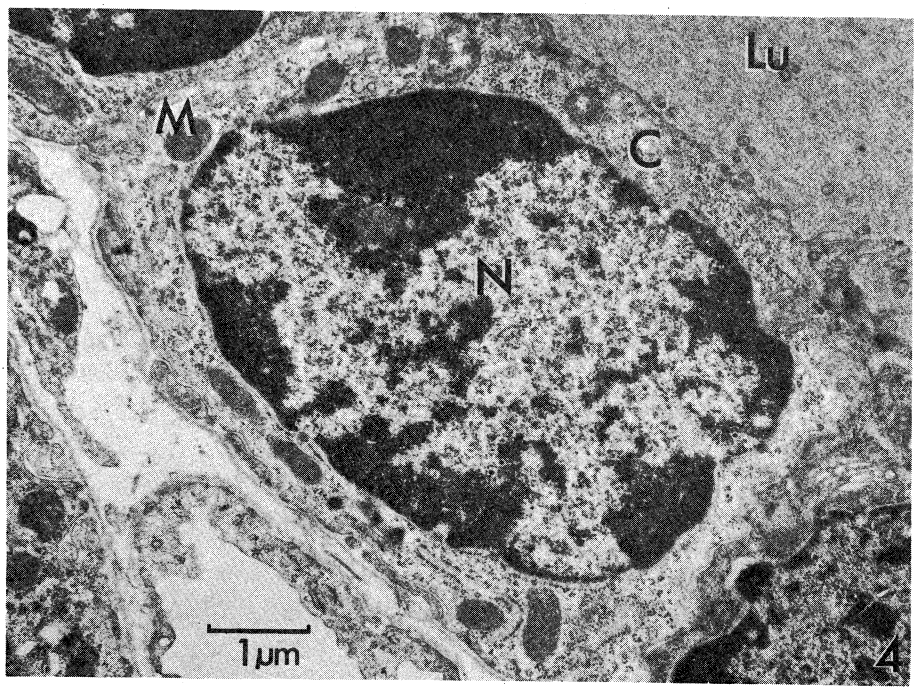


Fig. 3. A typical follicle cell from the thyroid of a phenotypically normal control mouse. The cytoplasmic inclusions include mitochondria (*M*), densely packed endoplasmic reticulum (*ER*) and lysosomes (*L*). The follicular lumen (*Lu*) can be seen in the top left-hand corner.

Fig. 4. A typical follicle cell from the thyroid of a dwarf mouse. Note the hypoactive mitochondria (*M*) and sparse endoplasmic reticulum in the small amount of cytoplasm (*C*) surrounding the nucleus (*N*). The follicular lumen (*Lu*) can be seen in the top right-hand corner.

Discussion

In the present study it was found that the plasma thyroxine concentrations were significantly lower in the dwarfs, being only one-eighth of that in control animals. That there was neither an age nor a sex difference in the plasma thyroxine concentrations of the dwarf mice has not been previously reported. Although serum thyroid hormone binding was also seen to be significantly lower in both age groups of each sex in dwarf mice compared with that of control groups, it was also found that the FTI was significantly lower in the dwarfs. The FTI is a convenient index of thyroid function because it compensates for differences in serum thyroid hormone binding as well as differences in total hormone and gives an indication of the free hormone available to the animal's tissues. The extremely low FTI in the case of the dwarf mouse thus reveals that the already small amount of thyroxine available in the dwarf plasma is effectively decreased further by the presence of a large amount of serum thyroid hormone binding capacity. The dwarf animal seems to be unable to compensate adequately for the lack of thyroxine by lowering its serum thyroid hormone binding sufficiently, leaving very little free circulating thyroxine available for the demands of the animal's tissues.

The total thyroid follicle size was found to be smaller with the thyroid follicle cells themselves being of reduced size and fewer in number in the dwarf mouse. The ultrastructure of the follicle cells revealed that the cells were hypoactive with little development of the endoplasmic reticulum and Golgi bodies. This is in accordance with the present findings of low circulating plasma T_4 concentrations. The nucleocytoplasmic ratio was significantly higher in dwarf thyroid follicle cells and this was seen to be due to the extremely hypoactive state of the cytoplasm which occupied a smaller volume within the cell.

Cell and nuclear diameters in the present study were in close agreement with those measured by Bartke (1964). Bartke (1965) suggested a primary defect in the functioning of the thyroid gland of the Snell dwarf mouse. From the present ultrastructural study of the thyroid and the findings of low T_4 concentrations in the plasma of the dwarf mouse it appears that either TSH is not present in adequate concentrations or that the thyroid gland does not respond to its presence. It has been reported, however, by Bartke (1965) and also observed by Howe and Pollard (unpublished work) that pituitary thyrotrophs are few in number and not in a very active state in dwarf mice. Measurements of TSH also have been extremely low in dwarf mice and TSH secretion did not occur when dwarf mice were administered TRH (Roti *et al.* 1978). Hence it seemed that the observed thyrotroph hypoactivity was not of hypothalamic origin. It seems therefore that a pituitary deficiency is responsible for the observed hypoactive thyroid gland and the low circulating levels of thyroxine in the dwarf. This possibility is currently being investigated.

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