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Morphometric study on the uterine horn and thyroid gland in hypothyroid, and thyroxine treated hypothyroid rats

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ABSTRACT

A wide range of reproductive disorders such as irregular menstruation and frank infertility is found in women with hypothyroidism. Most research done on these patients has focused on steroid and gonadotropin hormone profiles, whilst there has been little work on uterine morphology. Studies on hypothyroid animals have also demonstrated increases in fetal wastage, but there have been few studies of uterine structure in the hypothyroid rat. The present study has used hypothyroid Wistar rats as a model for investigating the effects of hypothyroidism on uterine structure. Three groups of Wistar rats were studied. One was made hypothyroid with methimazole (MMI), the 2nd was also made hypothyroid with methimazole but in addition the rats were simultaneously given daily thyroxine intraperitoneally (MMI+T4), and the 3rd was an untreated euthyroid group (control). Daily vaginal smears were obtained from rats in all 3 groups. All rats were aged 6 wk at the start of treatment and were killed after a further 6 wk. Uterine horns were removed and studied. Systematic random transverse sections were obtained from the proximal, middle, and distal regions of the horn and subjected to morphometric analysis. Difference between regions was assessed using 2-way analysis of variance. Absolute volume of endometrium in the uteri of hypothyroid rats was reduced by 45.1% ($P < 0.05$), whilst that of the muscle layer was decreased by 33.6% ($P < 0.05$). The cross-sectional area and absolute volume of the uterine horns were also reduced in hypothyroid animals ($P < 0.05$). In hypothyroid rats given thyroxine (MMI+T4) all variables increased significantly above those of hypothyroid rats. These changes suggest that hypothyroidism has an effect on uterine structure, which demonstrably improves under exogenous thyroxine administration. The observed structural changes might well play a significant role in the reproductive difficulties observed during hypothyroidism.

Key words: Infertility; endometrium.

INTRODUCTION

Hypothyroidism is a well documented cause of subfertility and debilitating menstrual disturbance in women (Hemady et al. 1978). Hypothyroid women seldom conceive and bear children (Kennedy & Montgomery, 1978; Boyland & Drury, 1979; Montoro et al. 1981). Indeed, pregnancy in hypothyroidism is so rare that it is considered a great achievement for a hypothyroid female to conceive (Balen & Kurtz, 1990). Clinical experience has shown that a wide range of menstrual disturbance is associated with hypothyroidism, ranging from total amenorrhoea to menorrhagia (Ramsay, 1986).

Even if a hypothyroid woman does conceive, the pregnancy itself is often very difficult to maintain successfully to term without complication. This is evidenced by a high incidence of spontaneous recurrent abortion, and of still births amongst hypothyroid pregnant women (Burrows, 1972; Ritchie, 1986). Experiments have indicated that hypothyroid animals also have difficulty in maintaining pregnancy. Studies in rats artificially made hypothyroid with propyl thiouracil have revealed increases in fetal resorption and a raised mortality at birth, as compared with control animals (Krohn & White, 1950). All these findings suggest a role for thyroid hormones in conception and maintenance of pregnancy. It is still not clear at which level of the hypothalamic–pituitary–ovarian–uterine axis altered thyroid function is most strongly expressed. Most research in man has been directed at the effects of hypothyroidism on

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the serum levels of gonadotropin and steroid hormones (Distiller et al. 1975; Heyburn et al. 1986; Fish & Mariash, 1988). There have been considerably fewer studies on uterine structure in this condition (Imamura, 1982).

The present study was carried out with the aim of establishing, by morphometric methods, whether (1) there is any distinction between the structure of the rat uterine horn in various regions, and, (2) if there are effects of hypothyroidism on uterine horn structure.

MATERIALS AND METHODS

Animal model

Eighteen 6-wk-old virgin female Wistar rats were used. They weighed between 150 and 200 g at the date of use. The animals were subjected to a constant cycle of 12 h light and 12 h darkness, and the temperature was maintained at 19–21 °C, with a relative humidity of 45%–55%. The rats were fed normal rat diet (Argo Foods, Penistone). Food and water were given ad libitum. At the end of 1 wk, the rats were divided randomly into 3 groups of 6 (A, B, C).

Group A (MMI) rats were made hypothyroid by replacing their drinking water with a solution of 0.02% w/v methimazole (Sigma UK), an antithyroid agent (Kirby et al. 1992; Meisami et al. 1992; Hardy et al. 1993). According to Silver & Leonard (1985), this is the minimum concentration at which thyroid activity is completely suppressed.

Group B (MMI + T4) rats were also given the same solution of 0.02% w/v methimazole in drinking water, but in addition were given a daily intraperitoneal injection of 5 μg thyroxine (Sigma) in 0.9% NaCl and 0.01 M NaOH.

Group C (control) rats were euthyroid animals maintained on tap water. Vaginal smears were taken daily from rats in all groups to determine the stage of oestrus cycle, whilst all rats were weighed once weekly. The start of the experiment (day 0) was when all rats in the 3 groups were in the oestrus stage. At the end of 6 wk (42 d), the control rats were in metoestrus stage. They were therefore killed 3 d later (i.e. at the oestrus stage). Treatment was started in the other 2 groups when the rats were also in oestrus. At the end of 6 wk (42 d) of treatment, hypothyroid rats were in dioestrus stage whilst thyroxine treated hypothyroid rats were in metoestrus stage. At this point, treatment was stopped, and hypothyroid and thyroxine treated hypothyroid rats were killed 3 d later when both groups were at oestrus. The oestrus cycle itself was irregular in hypothyroid rats. The rats in each group were weighed every week up to wk 6, and then on the day they were killed. A graph of the percentage of days spent at each stage of the cycle by all the rats in individual groups was drawn (see Fig. 4). At every weight measurement, the mean for each group was calculated (see Fig. 3). The length of the uterine horns of each was measured in situ before being removed, whilst the animals were alive. The 2 lobes of the thyroid gland were also removed and weighed wet to the nearest milligram, using an electronic balance (Sauter GmbH D-7470).

Tissue processing and sampling

The whole uterus was fixed in cold 4% phosphate buffered formaldehyde (pH 7.2, 380 mOsmol) for 24 h at 4 °C. From each rat one uterine horn was randomly selected. Randomness of selection was ensured as we had no idea from which side the horn came after fixation. In any case, in an earlier pilot study using a 2-way ANOVA, we had observed that there was no significant difference in the mean volume density of compartments in the different regions of the same uterine horn, or indeed between horns in the same animal (see Table 1).

Table 1. *Two-way ANOVA of Vv measurement of uterine horn luminal epithelium between regions of the same horn in euthyroid rats*

	Variation between regions			Variation within region				
Rat	SS	DF	MS	SS	DF	MS	*F*	*P*
1	2×10^{-5}	2	1.3×10^{-5}	2.7×10^{-4}	27	1×10^{-5}	0.9	0.3
2	2×10^{-5}	2	1.1×10^{-5}	5×10^{-4}	27	2×10^{-5}	0.5	0.6
3	4×10^{-5}	2	2.3×10^{-6}	8×10^{-4}	27	3×10^{-5}	0.8	0.4
4	4.6×10^{-5}	2	2.3×10^{-6}	2.7×10^{-4}	27	1×10^{-5}	2.3	0.1
5	2.6×10^{-5}	2	1.3×10^{-6}	2.7×10^{-4}	27	1×10^{-5}	1.3	0.2
6	2.6×10^{-5}	2	1.3×10^{-6}	5×10^{-4}	27	2×10^{-5}	0.7	0.5

SS, sum of squares; DF, degree of freedom; MS, mean squares; F = MS between regions/MS within region.

Each uterine horn was then divided into 3 equal regions by length; proximal, middle and distal. Each region was in turn cut transversely into 2 mm lengths, and after a brief wash in phosphate buffer (pH 7.2), the pieces were dehydrated through ascending concentrations of ethanol, and finally embedded in JB4 resin (Polysciences Inc.). Two blocks per region were selected randomly (by lottery) so that a total of 6 blocks was selected per rat. Ten nonconsecutive 2 μm random transverse sections were obtained from each block on an LKB Historange 2218 microtome using Ralph glass knife (Fig. 1). Randomness of location of sections was achieved by letting the block face be at an unknown distance from the knife edge; the block was then advanced until the first section was cut. Thereafter, sample sections were selected after every 50th section. The sections were collected with forceps and flattened by transferring them onto a water bath surface at room temperature. Sections were then collected on precleaned glass slides and air-dried before being stained with acid fuchsin and toluidine blue.

The thyroid gland was processed in similar manner to the uterine horn. Serial sections of 2 μm thickness were cut through the whole gland and 1 section was selected after every 100 sections. The 1st section was obtained by letting the block face be at an unknown distance from the knife edge, the block then being advanced until the 1st section was cut. Thicknesses of sections were accurately measured (mean thickness, 1.81 ± 0.07 μm) with a Vickers M86 scanning microinterferometer (Williams, 1977).

Morphometry

The absolute volume of the thyroid gland was estimated using the Cavalieri principle (Gundersen & Jensen, 1987). Each of the selected sections of the gland was projected at $\times 13$ magnification onto a sheet of white paper bearing a square lattice (square side 10 mm, equivalent to a test point area value on the sections of 0.5917 mm^2). Tracings of the outlines of the gland were made with a pencil. The area of each section was estimated and summed over all the sample sections. This area was used to estimate the absolute volume of the gland.

The sample sections of uterine horn and thyroid gland were viewed at a final magnification of $\times 100$ under a light microscope fitted with an eyepiece reticule (Graticules Ltd). The reticule had an array of test points (areal value of one point = $2.5\ 10^{-3}$ mm^2). A raster of systematic random fields of view was obtained by advancing the slide horizontally at equal intervals, beginning from an external point at which the tissue was not in view. Thereafter, the section was moved at equal intervals whilst counting the points hitting the various components of the tissue, until the opposite edge of the section was passed. The slide was moved vertically to a new alignment and the process of field placement and counting horizontally resumed moving in the opposite direction. This procedure was carried out until the whole section had been covered. The cumulative mean volume density estimates were plotted against the number of slides, so as to determine the minimum number of sections that would give a stable estimate of volume density (Williams, 1977).

Using computer software for linear measurements, the height of epithelium of thyroid follicles were measured. On the sample sections from the thyroid gland, the height of one epithelial profile was measured in a total of 100 follicles. Using the same software, the thickness of the endometrium from the luminal surface to the stroma–muscle border on the lateral sides of the horn was measured.

Using a projecting microscope, the luminal perimeter, and section profile area of uterine horn were estimated. This was carried out by projecting the sections at a final magnification of $\times 13$ onto a sheet of white paper bearing a square lattice (square side 10 mm, equivalent to a test point area value of 0.5917 mm^2). Tracings of the outlines of the lumen and of the external surface of the horn were made with a pencil. The intersections made by the luminal tracing with the test lines were used to estimate the luminal perimeter (Smith & Guttman, 1953), whilst the numbers of points overlying the horn tracing were used to estimate section profile area. An earlier pilot experiment had confirmed that there was insignificant difference in the mean transverse profile area between the 3 regions of the horn. Thus the absolute volume of each horn of the rat was estimated from the mean profile areas of sections together with the wet length of horn.

Statistical analysis

For each data set the arithmetic means, and coefficients of variation (C.V. = standard deviation as a percentage of corresponding mean) were computed. Comparison of parameters between horn sites (proximal, middle, and distal), and within animal groups, were drawn using a 2-way ANOVA (Table 1). Differences in parameters between animal groups were analysed using the nonparametric Mann–

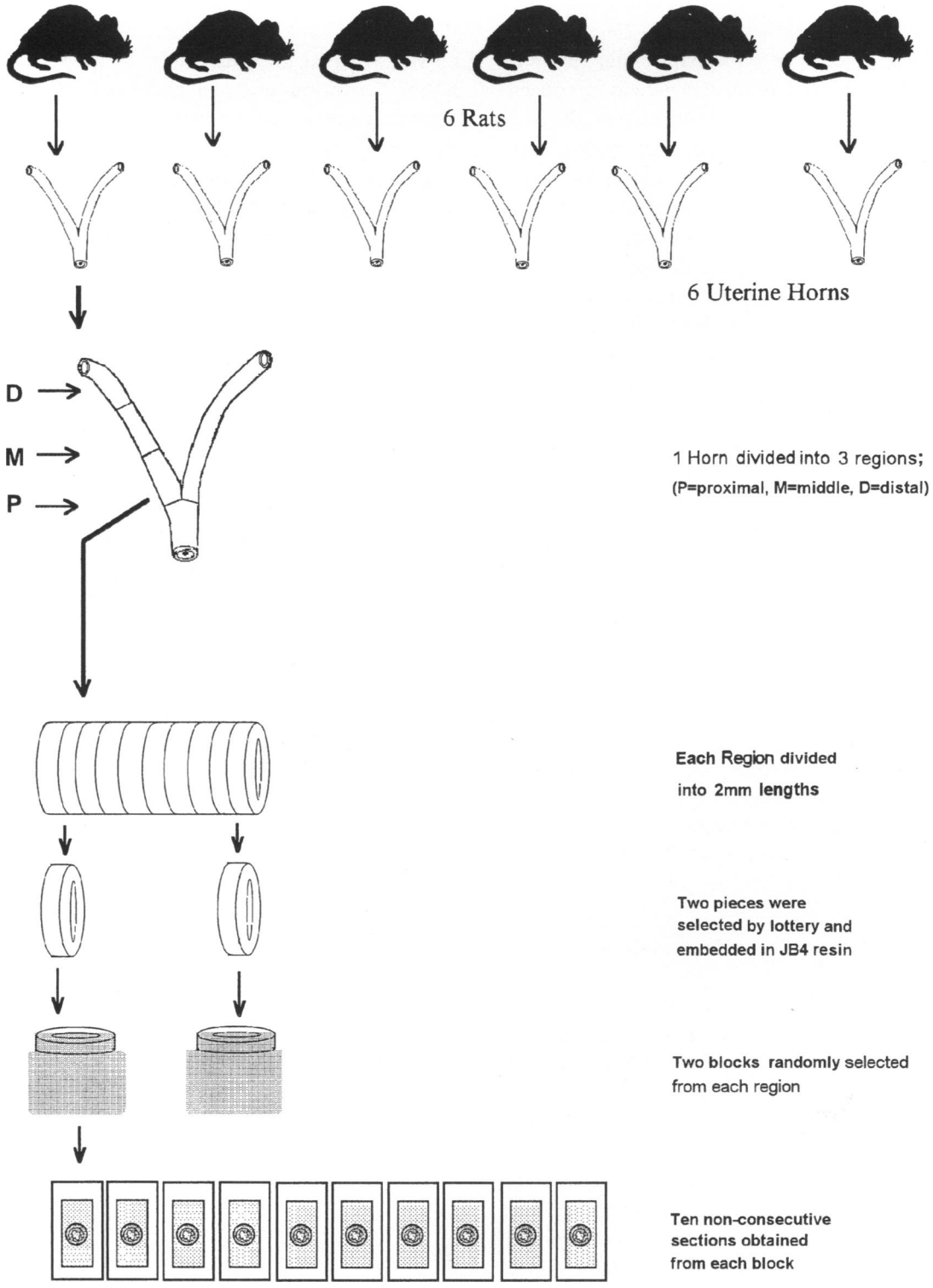


Fig. 1. Tissue sampling for morphometric study. Each uterine horn was divided into 3 equal regions, and each region further cut transversely into smaller pieces that were embedded in JB4 resin. Two blocks were randomly selected by lottery from each region making a total of 6 blocks per rat, and 10 nonconsecutive sections were obtained from each block.

Whitney U test. In each case, the null hypothesis was rejected if the probability of no difference was found to be less than 5% (i.e. $P < 0.05$). All statistical computations were made using the statistical package Instat, version 1.15 (Graphpad Softwares, 1990) on a personal computer.

Table 2. *Mean (CV%) relative and absolute volumes of different compartments of the thyroid gland in euthyroid (control), hypothyroid (MMI), and hypothyroid rats given thyroxine (MMI+T4)*†

Compartment	Volume density Vv			Absolute volume (mm^3)		
	Control	MMI	MMI+T4	Control	MMI	MMI+T4
Thyroid gland	—	—	—	34.72* (8)	81.59 (5)	56.88** (8)
Epithelium	0.41* (12)	0.56 (9)	0.49** (10)	14.23* (10)	45.69 (7)	27.87** (12)
Stroma	0.36 (20)	0.39 (5)	0.38 (17)	12.5* (10)	31.82 (5)	21.61** (13)
Colloid	0.23* (10)	0.06 (10)	0.13** (21)	7.98* (12)	4.89 (8)	7.39** (13)

† Results given are group means (CV%) for 6 rats. Activity index is the ratio of volume density (Vv) of follicular epithelium to that of the lumen (colloid) of the gland. Mitotic index is the number of mitotic figures per 1000 sectioned nuclei. Control, euthyroid; MMI, hypothyroid; MMI+T4, hypothyroid rats given thyroxine; * $P < 0.05$, euthyroid vs hypothyroid; ** $P < 0.05$ hypothyroid vs hypothyroid rats given thyroxine.

RESULTS

Thyroid gland

The results of the morphological assessment of the thyroid gland are illustrated in Tables 2 and 3.

Both weight and absolute volume of the thyroid gland were increased in hypothyroid (MMI) rats. Gland weight and volume in hypothyroid rats given thyroxine (MMI+T4) were also increased, but were significantly lower as compared with the hypothyroid (MMI) rats. In hypothyroid rats the volume density and absolute volume of thyroid follicular epithelium was increased significantly as compared with those of euthyroid (control), or of hypothyroid rats given thyroxine (MMI+T4), whilst those of the lumen (colloid) were decreased significantly. The volume density of the stroma remained unchanged in all groups, although its absolute volume had increased in hypothyroid rats as a result of the overall increase in the gland volume.

Thyroid follicular epithelial height (from basement membrane to surface) increased significantly in hypothyroid rats. The epithelial height in hypothyroid rats given thyroxine (MMI+T4) was not significantly increased when compared with euthyroid rats. Numerous mitotic figures were seen in the thyroid gland of hypothyroid rats, there being significantly fewer mitotic figures in the euthyroid rats. The ratio of the volume density of follicular epithelium to that of the colloid (Activity Index) was greatly increased in hypothyroid animals. A similar ratio was obtained when absolute volumes (instead of volume density) of the 2 compartments were used (see Tables 2 and 3).

Panels *A* and *B* of Figure 2 show the structure of thyroid gland in euthyroid (control) and hypothyroid (MMI) animals. Thyroid glands in euthyroid rats have follicles of various sizes, whose walls are made up of cuboidal or squamous follicular cells, depending on the state of activity of the follicle, which surround the colloid present in the lumena. Numerous blood vessels were present in the stroma, as is typical of any endocrine gland.

In hypothyroid rats the follicular nature of the thyroid gland was disrupted. The cuboidal follicular cells had become columnar, obliterating the lumina of the thyroid follicles, and the lumen (colloid) had almost totally disappeared. There were mitotic figures amongst follicular cells, a feature not commonly seen in the gland of euthyroid animals (see Fig. 2*a*, *b*).

Table 3. *Morphometric indicators of thyroid function in euthyroid (control), hypothyroid (MMI), and hypothyroid rats given thyroxine (MMI+T4)*†

Variable	Control	MMI	MMI+T4
Gland weight (mg)	30.8* (7)	82.5 (9)	59.75** (12)
Height of follicular epithelium (μm)	4.28* (42)	12.52 (38)	5.12** (52)
Activity index, AI (see text)	1.47* (10)	11.47 (10.7)	3.29** (18.2)
Mitotic index	0.95* (47)	16.8 (14.1)	1.65** (36.3)
Specific gravity of gland	0.901* (10)	1.03 (5.5)	1.05 (10.9)

† For explanation of abbreviations and symbols, see footnote to Table 2.

Changes in weights of rats and the oestrus cycle

Mean changes in weight during treatment amongst the 3 groups of rats are illustrated in Figure 3.

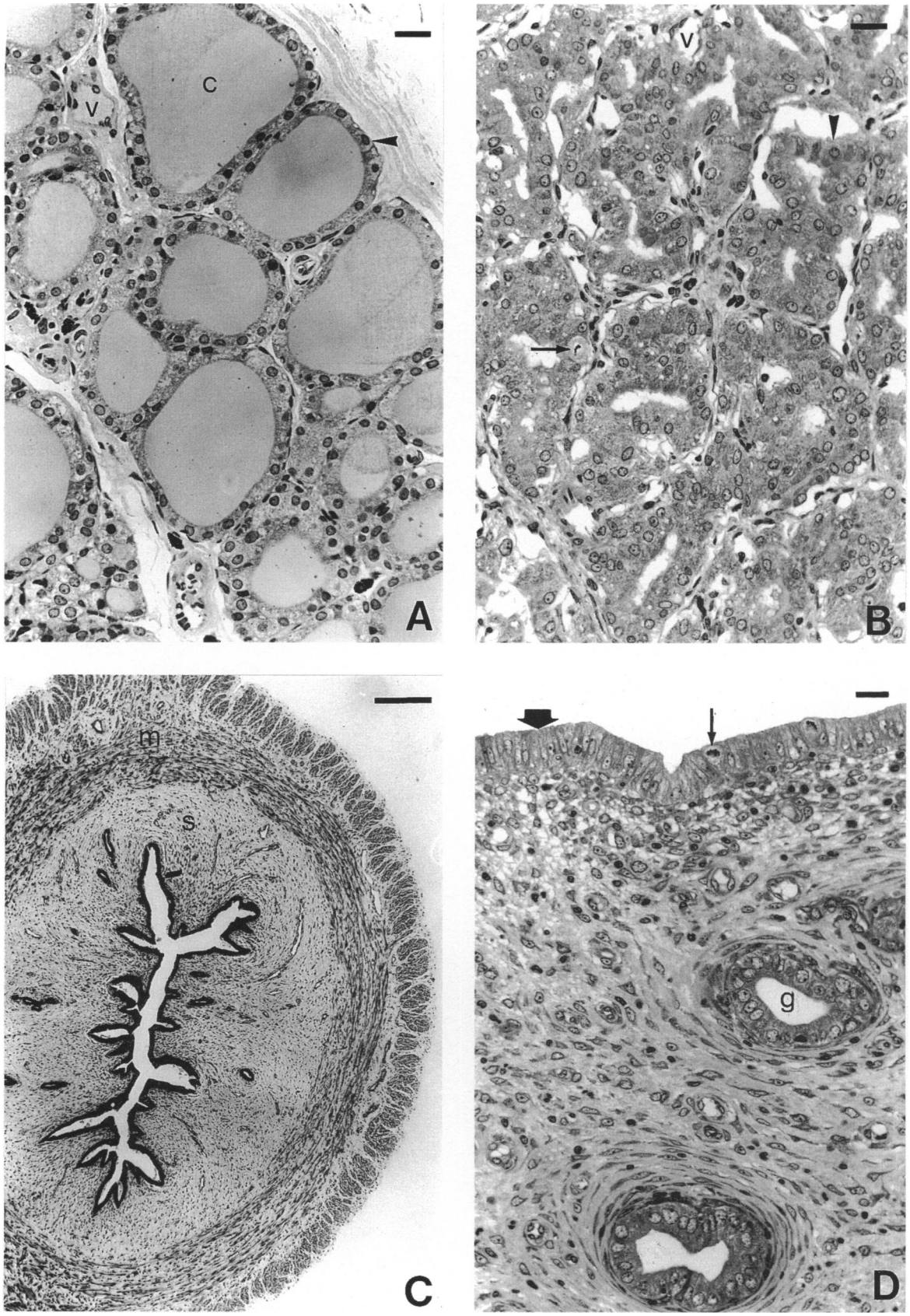
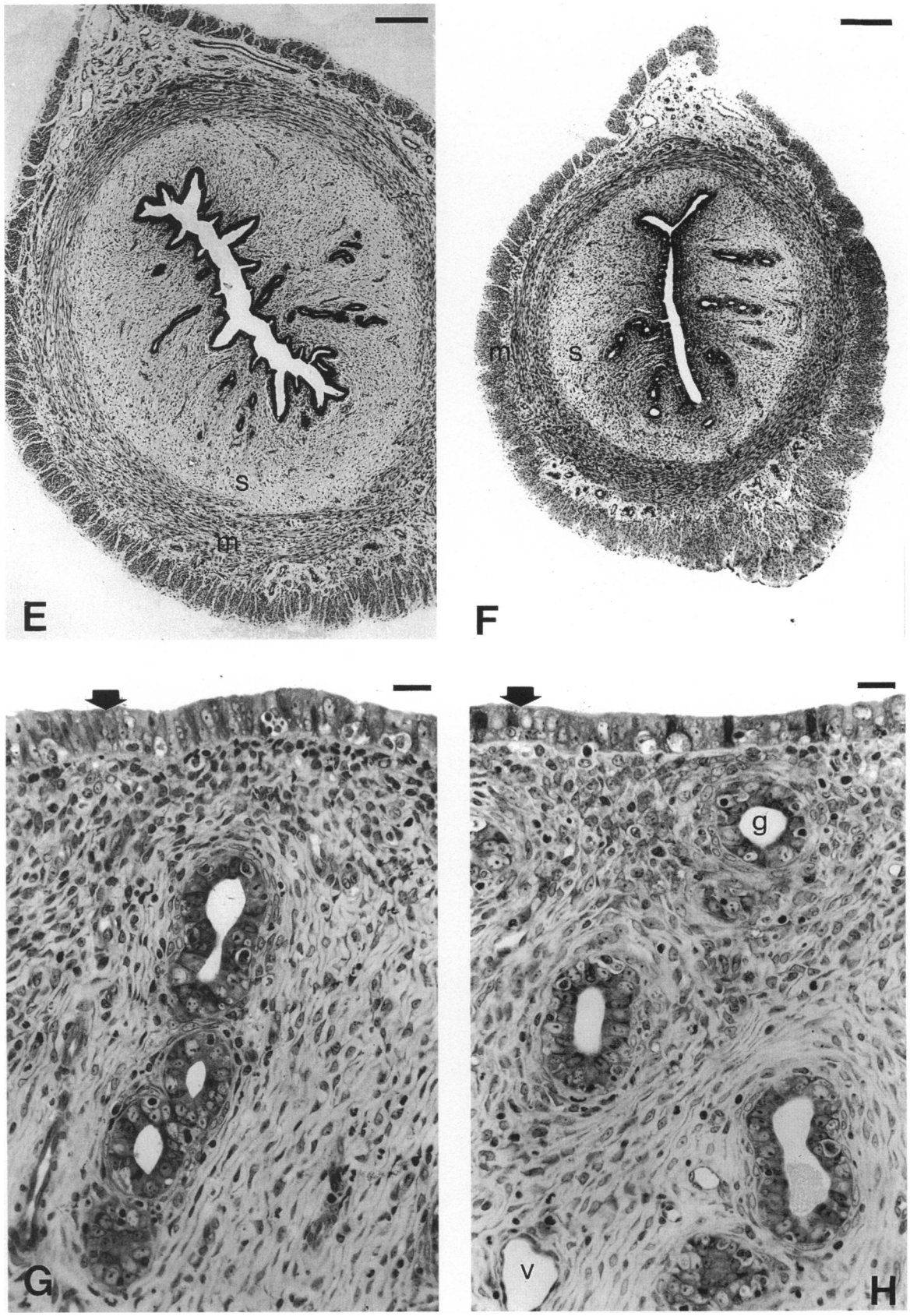


Fig. 2. (*A*) Normal rat thyroid gland composed of follicles of various sizes surrounded by cuboidal follicular cells (arrowhead). The lumina of the follicles contain colloid (c) rich in thyroglobulin. Blood vessels (V) were numerous. Bar, 20 μm. (*B*) Thyroid gland from methimazole treated rat. The follicular architecture of the gland is much disrupted. There is an almost total absence of colloid in the lumina of the follicles. Follicular cells (arrowhead) became columnar, and mitotic figures (arrow) were common amongst them. Bar, 20 μm. (*C*) Middle region of uterine horn (euthyroid). The horn consists of a columnar surface epithelium, a stroma (s) containing tubular glands, and circular and longitudinal muscle layers (m). The surface epithelium is thrown into folds, thereby increasing the luminal perimeter. Bar, 20 μm. (*D*) High



power view of the uterine horn in (*C*) showing columnar surface epithelium (short arrow). Mitotic figures (long arrow) are also commonly seen amongst the epithelia. g, gland. Bar, 20 μm. (*E*) Middle region of uterine horn in hypothyroid rat given thyroxine (MMI + T4). Note also the folded nature of surface epithelium here as in (*C*). Bar, 200 μm. (*F*) Middle region of uterine horn in hypothyroid rat. Note the flattened surface epithelium. s, stroma; m, muscle layer. Bar, 200 μm. (*G*) High power view of the uterine horn in (*E*) showing columnar surface epithelial cells (short arrow). Bar, 20 μm. (*H*) High power view of the uterine horn in (*F*). The surface epithelial cells (short arrow) are shorter than in (*D*) and (*G*) above. g, stromal gland; v, blood vessel. Bar, 20 μm.

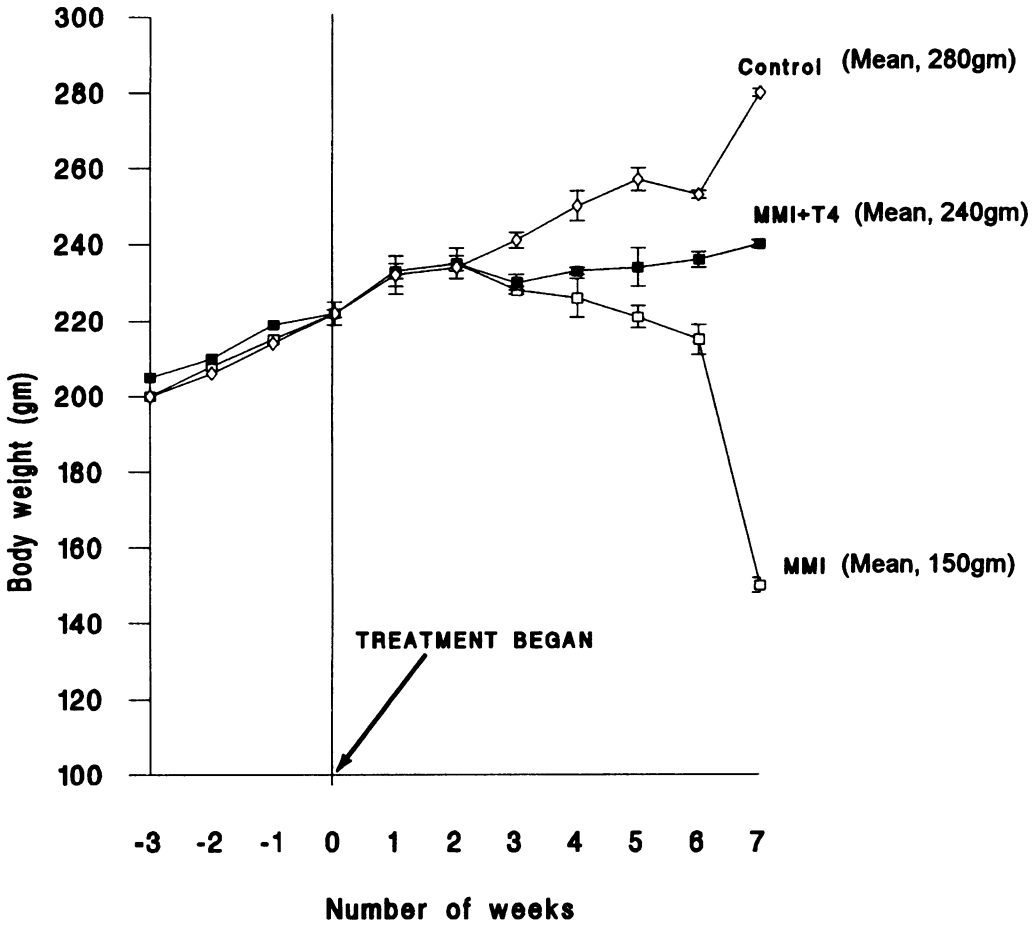


Fig. 3. Body weight and body weight change (mean ± S.E.M.) in euthyroid (control), hypothyroid (MMI) and hypothyroid rats given thyroxine (MMI + T4) during the experiment. Rats in each group were weighed weekly and the mean ± S.E.M. weight calculated.

Hypothyroid rats progressively lost weight, developed dry fur, and were observed to be less active than euthyroid (control) or hypothyroid rats given thyroxine (MMI + T4). Their weight loss was more pronounced from the 4th week of treatment (see Fig. 3). Hypothyroid rats were found to have disrupted oestrus cycles with a large proportion of the days (40.0%) spent in dioestrus, whilst only 12.3% was spent in oestrus. The euthyroid rats spent 26.3% of the days in oestrus stage with fewer days (22.7%) in the dioestrus stage of the oestrus cycle (see Fig. 4).

Uterine horn morphology

The uterine horn is made up of 3 distinct structural entities: the luminal epithelium, the stroma and 2 layers of smooth muscle. The stroma is composed of blood vessels and connective tissue cells. The luminal epithelium and stroma are collectively referred to as the endometrium. There is an inner circular and an outer longitudinal smooth muscle layer. A thin layer of connective tissue and mesothelium covers the external surface of the longitudinal muscle layer (see Fig. 2*c*, *e*, *f*). The luminal epithelium is simple columnar and rests on a thin basement membrane. It is thrown into folds, thereby increasing the surface area of the lumen (see Fig. 2*d*, *g*, *h*).

Uterine horn morphometry

The morphometric parameters determined in the uterine horn were the absolute volume of the uterine horn, the relative and absolute volumes of various compartments of the horn, thickness of the endometrium, and the length of the uterine horn. The results are given in Tables 2 and 3. The volume density of endometrium was significantly greater in euthyroid and hypothyroid rats given thyroxine (MMI + T4) when compared with that of hypothyroid rats. The cross-sectional area of the uterine horn was also significantly reduced in hypothyroid rats. The absolute volume of the uterine horn was reduced in hypothyroid rats as compared with euthyroid ones ($P < 0.05$). However, in hypothyroid rats given

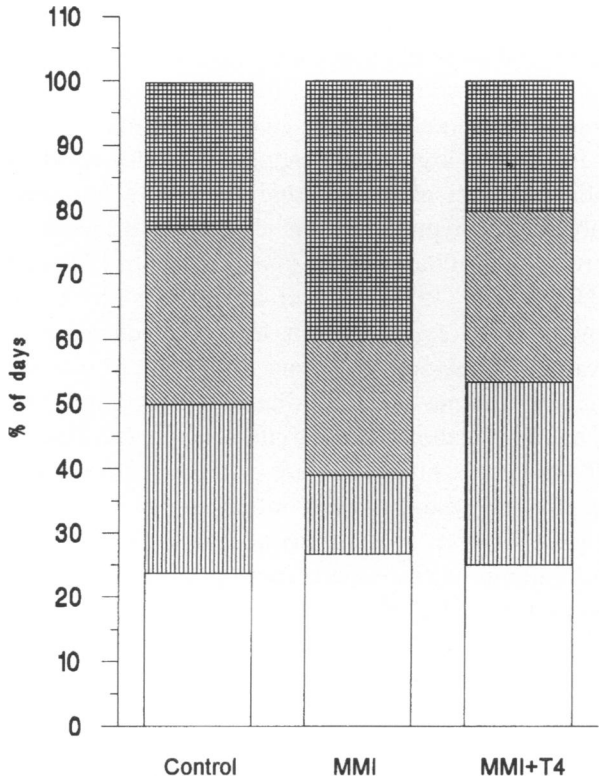


Fig. 4. Mean percentage of days spent on various stages of the oestrus cycle by each group of rats. Control, euthyroid; MMI, hypothyroid; MMI+T4, hypothyroid rats given thyroxine. The stage of oestrus cycle was determined by daily vaginal smears. ▦ = dioestrus, ▧ = metoestrus, ▥ = oestrus, □ = proestrus.

thyroxine (MMI+T4), the volume was significantly higher ($P < 0.05$) as compared with hypothyroid rats. The decrease in volumes of different compartments of the uterine horn in hypothyroidism appear to be nonuniform. Whilst the endometrial volume had decreased by 45.1%, the muscle volume had decreased by 33.6% (see Table 4).

The thickness of the endometrium from the luminal surface to the stroma-muscle junction was found to be

Table 5. *Morphometric variables for uterine horn in euthyroid (control), hypothyroid (MMI), and hypothyroid rats given thyroxine (MMI+T4)*†

Variable	Control	MMI	MMI+T4
Endometrial thickness (mm)	0.72*	0.54	0.62**
	(7.5)	(6.3)	(12.5)
Length of uterine horn (mm)	46*	42	44
	(6.5)	(8.5)	(7.1)
Cross-sectional area of horn (mm^2)	4.24*	2.91	3.55**
	(1.6)	(1.7)	(1.7)
Luminal perimeter (mm)	0.959*	0.725	0.857**
	(12.1)	(6.3)	(10.8)

† For explanation of abbreviations and symbols, see footnote to Table 4.

significantly greater in euthyroid rats than in hypothyroid rats ($P < 0.05$). Length of the uterine horn from its junction with the body of the uterus at one end, to the horn–fallopian tube junction at the other, as well as its cross-sectional area had similarly decreased in hypothyroid rats. However, the length of the uterine horn had only decreased by 8% whilst the cross-sectional area had decreased by 31.3% (see Table 5).

DISCUSSION

This study has demonstrated the occurrence of significant alterations of uterine morphology in hypothyroidism. The absolute volume of the uterine horn in hypothyroid animals was significantly reduced as was the wet weight of the organ. These changes were evidently partly prevented by thyroxine administration to hypothyroid animals.

The rarity of pregnancy in hypothyroid women has generally been attributed to a high incidence of

Table 4. *Mean (CV%) relative and absolute volumes of different compartments of the uterine horn in euthyroid (control), hypothyroid (MMI), and hypothyroid rats given thyroxine (MMI+T4)*†

	Volume density Vv			Absolute volume (mm^3)		
Compartment	Control	MMI	MMI+T4	Control	MMI	MMI+T4
Uterine horn	—	—	—	195.04*	122.22	156.2**
				(10.5)	(9.8)	(4.7)
Endometrium	0.32*	0.28	0.30**	62.41*	34.22	46.86**
	(3.1)	(5.3)	(9.8)	(8.0)	(12.1)	(9.5)
Muscle	0.67*	0.70	0.68**	132.63*	88.0	109.34**
	(5.9)	(6.4)	(7.7)	(5.9)	(8.8)	(11.2)

† Results are group means (CV%) for 6 rats. Endometrial thickness was measured from the luminal surface of horn to the stroma–muscle junction. Luminal perimeter was the length of the outline of luminal epithelium in cross section. Control, euthyroid; MMI, hypothyroid; MMI+T4, hypothyroid rats given thyroxine; * $P < 0.05$, euthyroid *vs* hypothyroid; ** $P < 0.05$ hypothyroid *vs* hypothyroid rats given thyroxine.

anovulation (Goldsmith et al. 1952). The observations made here suggest that, in addition to anovulation, altered uterine morphology may play a part in preventing pregnancy.

The hypothyroid rats exhibited physical features of hypothyroidism with a significant increase both in the weight and absolute volume of the thyroid gland. This increase was clearly due to both hypertrophy and hyperplasia of follicular cells as evidenced by an increase in their height as well as the presence of numerous mitotic figures. Hypothyroidism was further demonstrated by the morphometric assessment of gland structure which correlates quite well with the functional status of the gland as was suggested by Kalisnik (1981).

Normally functioning human and rat thyroid glands have follicles of various sizes whose walls are made up of cuboidal or squamous follicular cells. The lumen of each follicle contains thyroglobulin (colloid) which serves as a storage form of thyroxine. In long standing hypothyroidism caused by methimazole, the normal follicular structure of the thyroid gland is disrupted. The cuboidal follicular cells become columnar, obliterating the lumina of the follicles, and the colloid almost totally disappears. The specific gravity of thyroid gland is significantly increased in hypothyroid animals ($P < 0.01$) presumably due to a decrease in colloid content.

The improvement of uterine structure to near euthyroid values by thyroxine administration to hypothyroid animals suggests that in euthyroid animals the presence of the hormone contributes to the maintenance of normal uterine morphology. Indeed, DeCherney & Polan (1984) had already shown that patients suffering from habitual abortion caused by hypothyroidism respond favourably to thyroxine therapy. The decrease in uterine horn weight and volume in hypothyroid rats could be thus a direct effect, particularly since thyroid hormone is believed to exert its influence on tissues by facilitating the transcription of DNA resulting in new protein synthesis. Evans et al. (1983) and Mukku et al. (1983) have independently shown that the rat uterus is a specific site for thyroid hormone action by demonstrating the presence there of thyroid hormone receptors. It is known that thyroid hormone has a growth promoting action in rats, its absence leading to growth retardation (Evans et al. 1960). Absence of the hormone could thus slow down the normal processes of tissue growth and replacement resulting in a decrease in the size of the uterine horn. It appears, however, that the effect of hypothyroidism on the uterine horn was more pronounced on the endometrium than the smooth muscle layer. This could be as a result of unequal levels of metabolic activity, or due to a disproportionate distribution of thyroid hormone receptors in these 2 compartments.

It could be argued that changes may also occur as indirect effects of hypothyroidism since it has been shown that hypothyroidism leads to a decrease in serum levels of the pituitary gonadotrophins FSH and LH (Akande, 1975; Valle et al. 1985; Kirby et al. 1992). These could in turn lead to a decrease in ovarian stimulation and hence a decrease in ovarian steroid hormone levels, causing a drop in stimulation of uterine metabolism. These effects might also explain why there is a preponderance of dioestrus smears in the hypothyroid rats in contrast to euthyroid or hypothyroid rats given thyroxine (MMI + T4). In fact, our findings on this aspect agree with those of others (Vriend et al. 1987; Ortega et al. 1990) who also found a persistence of dioestrus smears and a reduction of oestrus smears in hypothyroid rats. As the dioestrus stage in rats is characterised by low serum levels of gonadotrophins, this observation suggests that hypothyroidism does have influence on the levels of these hormones.

This study therefore has provided quantitative data which suggest that the structure of the rat uterine horn was altered in hypothyroidism, and that this alteration was reversible with thyroxine administration. Further studies on the effect of hypothyroidism on protein metabolism and cell kinetics in the uterine horn, as well as ovarian enzyme histochemistry are being planned.

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