

قسم الولادة

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التغيرات المورفولوجية والقياسية في الغدة الدرقية لذكور الأرانب بعد حقنها بهرمون الجونادوتروبين كريوني البشري بروبيونات التستستيرون

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أجريت هذه الدراسة على عدد ٣٣ من ذكور الأرانب البالغة من سلالة البوسكات قسمت الى خمسة مجموعات واعتبرت المجموعة الأولى كضابط ثم حُقنت المجموعات الأخرى بجرعات مختلفة من الهرمون المشيمي الآدمي الحاث للمناسل البشري بروبيونات التستستيرون.

وقد دلت الدراسات القياسية على أن الجرعات المختلفة بالهرمون المشيمي الآدمي الحاث للمناسل أدت الى نقص ملحوظ في حجم الحويصلات الدرقية وكذلك حجم المواد الغروية لهذه الحويصلات .

مع زيادة ملحوظة في عدد الحويصلات الدرقية وكذلك زيادة في حجم الخلايا المبطننة هذا مما يدل على أن الجرعات المختلفة للهرمون المشيمي الآدمي الحاث للمناسل أدت الى زيادة في نشاط الغدة الدرقية . وقد لوحظ أيضا أن الجرعات العالية والمنخفضة من بروبيونات التستستيرون أدت الى زيادة ملحوظة في نشاط الغدة الدرقية .

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**MORPHOLOGICAL AND STEREOLOGICAL CHANGES IN THE THYROID  
GLAND OF RABBITS AFTER LONG-TERM HCG AND TP ADMINISTRATION\***  
(With 3 Tables and 7 Figures)

By

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**SUMMARY**

The effect of long-term administration of various doses of hCG or TP on the thyroid of 33 adult male rabbits of pure Bouscat breed were histologically and stereologically investigated. The thyroid activity exhibited a dose-related increase in rabbits injected with hCG. A marked progressive decrease in the follicular and colloidal volumes as well as gradient increase in the number of follicles per mm<sup>3</sup>, volume of the follicular cell, nuclear volume and stromal vascularization were observed in the thyroids of rabbits treated with hCG.

The low and high doses of testosterone propionate (TP) induced morphological signs of higher glandular activity without any significant difference between the doses of TP.

**INTRODUCTION**

The thyroid activity is altered after treatment with human chorionic gonadotropin (hCG) or testosterone propionate (TP). Recent studies demonstrated that hCG is a thyroid stimulator which was previously called molar thyrotropin (KENIMER *et al.*, 1975 and HERSHMAN, 1978). It possesses a larger molecular weight with a potency of about 1/4000 of pituitary TSH. Administration of testosterone or its congeners, in pharmacological dosage greatly decreased thyroxine-binding globuline (TBG). The total serum T<sub>4</sub> and T<sub>3</sub> were also declined to values similar to that of the hypothyroid range (BRAVERMAN and INGBAR, 1967). However, other studies have shown a consequent increase in the concentration of free serum T<sub>4</sub> after injection of testosterone (BELLABARBA *et al.*, 1968).

The lack of a decisive description for the histomorphological features of the thyroid glands particularly in rabbits, after hCG or TP manipulation in the available literature was the principle modulation which evoked our encouragement to carry out the present investigation.

It is worthy to mention that the present work is a further study concerning the effect of hCG and TP on the reproductive functions in adult rabbits.

**MATERIAL and METHODS**

Thirty three adult male rabbits (9 months old, approx. 2.5 Kg.) of pure Bouscat breed were purchased from the animal breeding farm at the University of Assiut. The animals were divided into 6 groups and treated as presented in table 1.

The drug were administered by deep intramuscular injection into the anterior of the thigh muscles. Animals were sacrificed during the 16<sup>th</sup> week after the commencement of

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treatment. The thyroid glands were immediately obtained after slaughtering, dissected into small pieces, fixed in Bouin's fluid and processed for histological examination. The sections were stained with Haematoxylin and Eosin, Periodic acid Schiff (PAS)- Haematoxylin (Mc MANUS, 1948) and Crossman's trichrome stain. The proportion of parenchymal and stromal components of the thyroid was determined by the aid of a square grid with 100 points mounted in the eye-piece of a light microscope (WEIBEL *et al.*, 1966). The microscopical measurements were made with eye-piece screw micrometer scale which was calibrated with a stage micrometer to the nearest micron. The follicular diameter, cell height and width as well as nuclear diameter were counted from 500 randomly selected follicles for each thyroid. The obtained data were statistically analysed according to SNEDECOR and COCHRAN (1967). The stereological methods are summarized in table 2.

## RESULTS

### Histological structure :

The thyroid gland of control rabbits (Fig. 1) is surrounded by a well defined connective tissue capsule from which septa extend into the interior of the gland, separating it into well distinct lobules. The follicles are separated by loose connective tissue stroma containing blood vessels, nerves and lymphatics. The thyroid follicles are rounded or oval in shape. They are lined with cuboidal cells and contain slightly acidophilic colloid substance. There are a discernible pattern in the distribution of the small and large follicles within the thyroid gland of rabbits. The largest follicles were observed in the peripheral lobules rather than in the central ones, however, some different gradient of follicles were also present. The mean follicular volume, number of cells per follicle in control rabbits are presented in table 3.

After treatment with various doses of hCG (Figs. 2,3,4), a dose related change in the thyroid follicles was observed. The thyroid follicles were gradually decreased in size, however, the follicular cells increased in height and contain large rounded nuclei (Fig. 5). After high dose of hCG, the apical portion of the follicular cells contained numerous intracellular droplets which were positively stained with PAS stain (Fig. 5). These droplets were probably represented the engorged luminal colloid.

After injection of low and high doses of testosterone propionate, a dose related changes were only demonstrated in the diameter of thyroid follicles (Figs.6,7), however, the follicles became smaller in size and lined with columnar cells containing larger vesicular nuclei. Moreover, the other histological features were essentially similar to that observed after injection of high dose of hCG.

### Stereological analyses (Table 3):

#### Human chorionic gonadotropin (hCG) :

After treatment with various doses of hCG, the mean follicular diameter and consequently the mean follicular and colloidal volumes showed statistically significant reduction in size than in control rabbits. However, the smallest volumes were recognised in animals treated with high dose of hCG. No dose related changes were observed between the low and medium doses of hCG. Nevertheless, the high dose of hCG resulted in reduction which was significantly greater than that caused by the low or medium doses. Therefore, the highest number of thyroid follicles per mm<sup>3</sup> was recognised after administration of high dose of hCG, considering that a dose related increase in the number of these follicles was observed after injection of the middle and high doses of hCG. The follicular cell volume showed a significant increase



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which was more pronounced for the high dose of hCG. Similarly after high dose of hCG, the nuclear volume of these cells showed statistically significant increase, although this was not dose related. In addition, the mean number of cells per follicle was significantly affected only in case of the medium and high doses of hCG. Within this group the change in the follicular cell height was increased with the increase in the dose of hCG.

#### Testosterone propionate (TP) :

After treatment with low or high dose of testosterone propionate (TP), the follicular diameter, therefore, the follicular and colloidal volumes were significantly decreased. In addition, this reduction in size was dose related. The follicular cell volume was significantly increased than that of the control animals, but without any effect of the dose. Otherwise, no significant difference was recorded in the mean number of cells per follicle after treatment with either low or high dose of TP. However, the mean nuclear volume of the follicular cells was significantly increased after TP injection and their values were about three times more than that of the untreated ones.

The proportion of the stromal to the parenchymal components of the thyroid gland was slightly increased after treatment with high dose of hCG and TP than in control rabbits. This stromal increment was probably due to an increased stromal vascularization.

### DISCUSSION

The results of the work herein indicated that the thyroid morphology depends mainly on the shape of the follicular cells, for the reason that they are concurrently involved in both synthesis and release of thyroid hormones (WARNER and INGBAR, 1978). Moreover, the stereometric analyses showed that the follicular and colloidal volumes are mainly influenced by the follicular cell volume. Recent studies have shown that the thyroid stimulator, in the hyperthyroid woman with hydatiform mole, is the human chorionic gonadotropin and it has a potency of about 1/4000 of the pituitary TSH (KENIMER *et al.*, 1975). The present findings demonstrated that the thyroid gland of rabbits exhibited a dose-related increase in their activities after injection with various increased doses of hCG. The morphological signs of the increased glandular activities were represented by a progressive increase in the mean volume of the follicular cells, progressive absorption of the luminal colloid, gradual increase in the number of follicles per mm<sup>3</sup> and increased stromal vascularization as well. Such findings may be attributed to the fact that hCG has a larger molecular weight with a longer duration of action than the hypophyseal TSH (HERSHMAN *et al.*, 1970).

The present results also revealed that administration of either low or high dose of testosterone propionate persuaded a significant increase in the thyroid activities, without any obvious morphological differences between the both doses of testosterone propionate. Although, the physiological interactions of thyroid hormones with the pituitary - testicular system have been documented, the occurrence of hyperthyroidism and male infertility is rare (HEMBREE, 1978). Moreover, BRAVERMAN and INGBAR (1967); BRAVERMAN *et al.* (1968) and HELLMAN & ROSENFELD (1974) mentioned that androgen therapy in man initially resulted in a striking decrease in thyroxine-binding globuline (TBG) with a subsequent decrease in the total serum thyroxine level. Recently, INGBAR (1985) stated that administration of estrogens or androgens has no consistent effect on Radio Active Iodine Uptake (RAIU), but causes alteration in the binding of thyroid hormones in plasma. Estrogens increase the concentration of TBG and elevate the serum T<sub>4</sub> and T<sub>3</sub> concentration, whereas androgens induce converse effects. These findings are greatly supported by the present data and it could be interpreted to mean that the decreased level of the total serum thyroxine stimulate the pituitary gland for increased



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release of TSH which in turn increase the thyroid gland activities. Thus it can be suggested that testosterone propionate may have an indirect stimulatory effect on the rabbit thyroid glands.

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Table (1)

## Materials available for study

Group	Number of rabbits	Treatment
Control	6	untreated
hCG (low)	5	200 I.U.hCG/day
hCG (Medium)	6	400 I.U.hCG/day
hCG (High)	5	800 I.U.hCG/day
TP (Low)	6	5 mg TP/Kg/day
TP (High)	5	10 mg TP/Kg/day

Testosterone propionate was obtained from Eli Lilly and Co. (Indianapolis, Ind. USA ) and later from Memphis Co. ( Cairo, Egypt ), hCG (Coriantin) was obtained from the Nile Co. (Cairo, Egypt).

Table (2)

## Stereological methods

Components measured	Evaluated parameters	Methods Applied	Reference
Parenchymal & stromal Components	Proportion	Point counting	Weibel <u>et al.</u> , 1966
Follicle	Area, volume,	Wicksell's transformation	Wicksell, 1925.
Colloid	" "	" "	" "
Follicular cells	Height, basal area, volume.	Cordier <u>et al.</u>	Cordier <u>et al.</u> , 1976.
Follicular cell nuclei	Volume.	Elias & Hyde 1980	Elias & Hyde 1980



Table (3)  
Stereological analyses of the thyroid gland in rabbits treated with hCG and Tp.

Group	Follicular diameter Um	Follicular volume Um <sup>3</sup> X10 <sup>3</sup>	No. of follicles/m <sup>3</sup>	Colloidal volume Um <sup>3</sup> x10 <sup>3</sup>	Follicular cell height Um	Nuclear volume Um <sup>3</sup>	Single cell volume Um <sup>3</sup>	Number of cell/follicle	Gland component	
									Stroma %	Parenchyma %
Control	86.09 +1.01	240.21 + 3.93	3840 +27	186.00 +1.64	6.38 +0.27	34.00 +1.211	196.00 +1.32	359.00 +2.16	20.00 +0.29	80.00 +0.29
hCG Low	71.56 <sup>a</sup> +0.50	155.09 + 3.03	6448 +15	77.16 +1.26	6.95 +0.10	52.00 <sup>a</sup> +0.26	216.00 <sup>a</sup> +1.45	361.00 +0.65	19.82 +0.27	80.18 +0.27
hCG Medium	70.18 <sup>a</sup> +0.25	139.56 +1.43	7165 +18	56.31 +0.76	9.25 <sup>a</sup> +0.18	52.00 <sup>a</sup> +0.36	225.00 <sup>a</sup> +1.59	370.00 <sup>b</sup> +1.90	22.00 +0.20	78.00 +0.20
hCG high.	61.23 <sup>a</sup> +0.18	119.89 +0.64	8341 +18	40.13 +0.66	10.40 <sup>a</sup> +0.25	55.00 <sup>a</sup> +1.27	265.00 <sup>a</sup> +2.05	377.00 <sup>a</sup> +0.84	23.00 +0.45	77.00 +0.45
Tp Low	65.58 <sup>a</sup> +0.20	124.28 +0.93	8046 +15	42.63 +0.38	9.85 <sup>a</sup> +0.18	95.00 <sup>a</sup> +2.24	227.00 <sup>a</sup> +1.34	360.00 +1.23	22.08 +0.22	77.92 +0.22
Tp high	58.70 <sup>a</sup> +0.20	123.71 +0.34	8032 +16	41.72 +0.31	9.80 <sup>a</sup> +0.20	95.00 <sup>a</sup> +3.16	226.00 <sup>a</sup> +1.27	362.00 +0.90	23.32 +0.24	76.68 +0.24

a = P &lt; 0.01

b = P &lt; 0.001



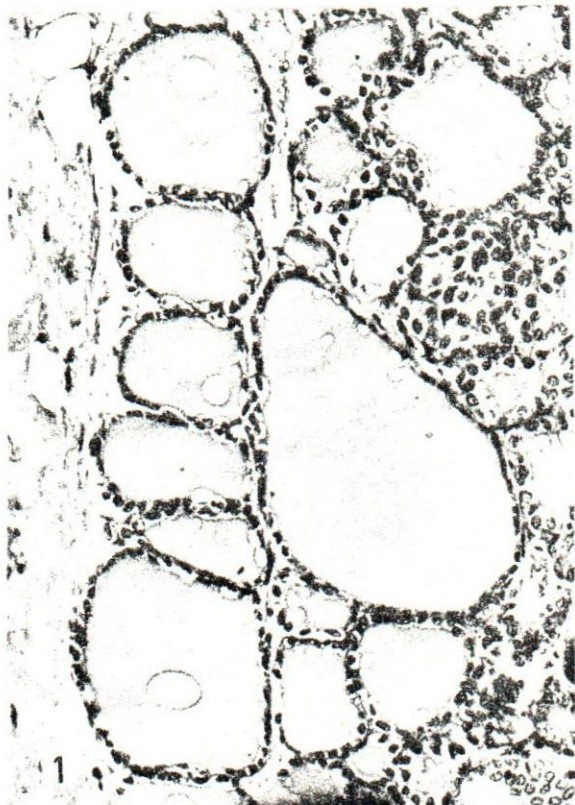


Fig. (1): Normal control rabbit thyroid follicles. (PAS & Hx. 160).

Fig. (2): Rabbit thyroid follicles treated with low dose of hCG. (PAS & Hx. X 160).

Fig. (3): Rabbit thyroid follicles treated with medium dose of hCG. (PAS & Hx. X 160).

Fig. (4): Rabbit thyroid follicles treated with high dose of hCG. (PAS & Hx. X 160)



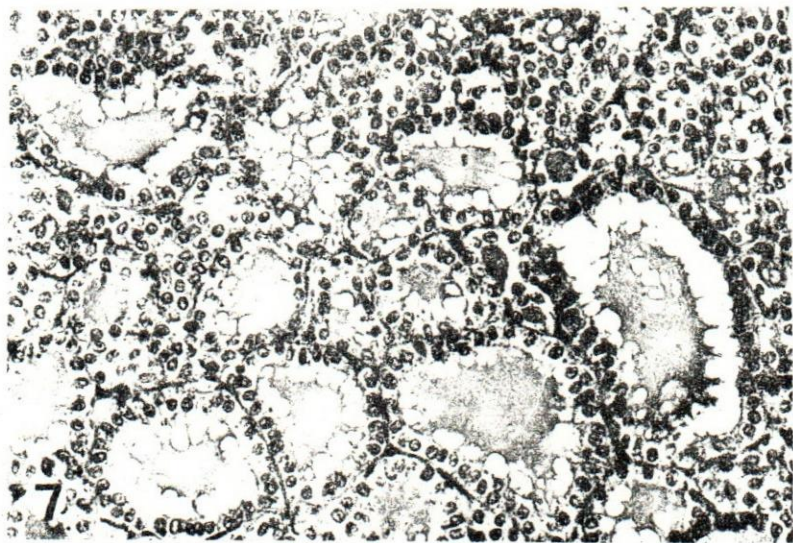
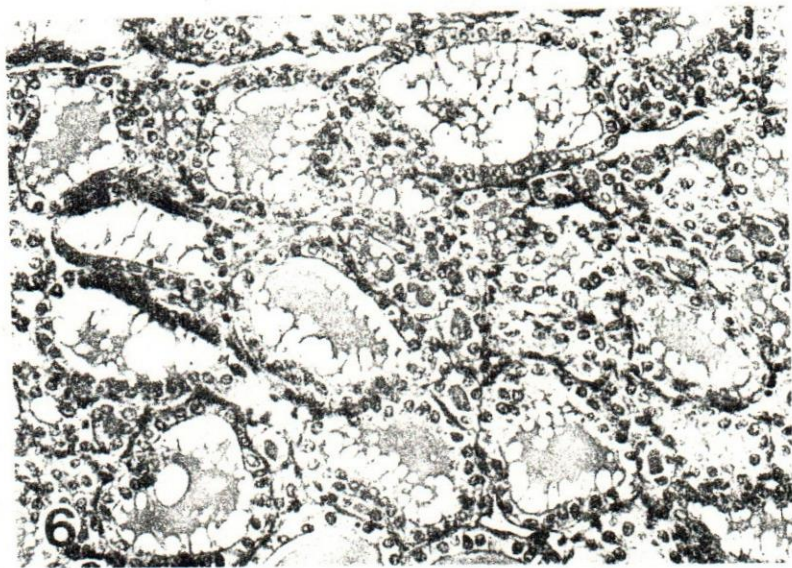
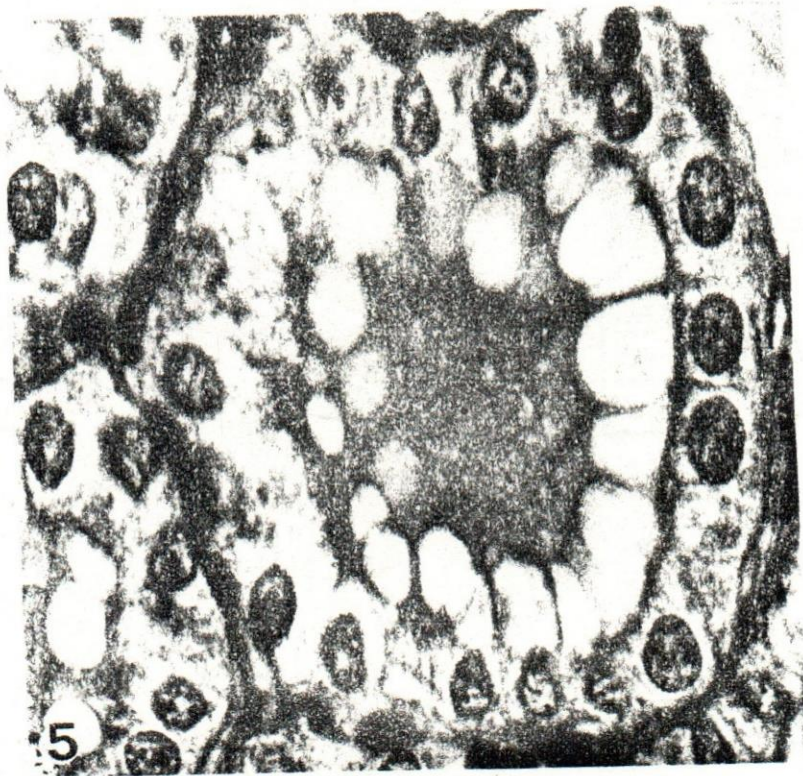


Fig. (5): Higher magnification of rabbit thyroid follicle treated with high dose of hCG, showing numerous intracytoplasmic droplets and engulfed luminal colloid. (PAS & Hx X 400).  
Fig. (6): Rabbit thyroid follicles treated with low dose of TP. (PAS & Hx. X 160)  
Fig. (7): Rabbit thyroid follicles treated with high dose of TP. (PAS & Hx. X 160).