EFFECT OF ETHINYL OESTRADIOL AND NORETHISTERONE ALONE OR IN COMBINATION ON THYROID FUNCTION AND HISTOLOGY OF THE OVARY, THYMUS, ADRENALS AND UTERUS*

By

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There are a number of reports on the effects of oestrogens or progesteron on thyroid function in experimental animals and humans (1, 2, 9). There are a few reports on the effects of combinations of oesterogens and progesterone on thyroid weight and histology. Fixed combinations of oesterogens and progesteron are extensively used as oral contraceptives. It was, therefore, decided to investigate the action of one such fixed combination on histology of target endocrine glands and thyroid function in rats.

MATERIALS AND METHODS

The experiments were performed in five groups of six female rats each, obtained from Ciba Research Centre, Goregaon, Bombay. The average weight of rats was 150 *g* and their age varied from 3 to 5 months. This age range was selected because rats are most fertile during this period.

All drugs were dissolved in olive oil and were given intramuscularly daily alternately in the thigh muscles of the two sides. The volume of injection did not exceed 0.2 *ml*. Group A was given 0.2 *ml* of olive oil and served as the control. Group B was injected norethisterone acetate in a dose of 50 $\mu g/kg$. Group C received ethinyl oestradiol in a dose of 1 $\mu g/kg$. Group D received the combination of norethisterone acetate and ethinyl oestradiol in the same proportion as existing in Norlestrin a commercial preparation marketed by Parke Davis and Company (50 μg of norethisterone/*kg* and 1 μg of ethinyl oestradiol/*kg*). Groups A to D received injections every day for a period of six weeks. Group E received injections of the combinations of above mentioned oestrogen and progesterone (1 : 50) over a period of seven weeks. At the end of these periods, injections were discontinued and the animals were allowed a recovery period of four weeks, after which they were all sacrificed at one time. Animals were weighed initially and at the end of the experiment before sacrificing.

Twenty-four hr after the last dose of the drug, each rat in groups A to D was administered I^{131} in a dose of 0.5 μc in 0.5 *ml*. The rats of group E, (the recovery group) received I^{131} forty-eight hr prior to the day of sacrifice.

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Forty-eight hr after injection of I^{131}, the rats were anaesthetised with ether, the ches was opened up and blood was collected by direct cardiac puncture for PBI^{131} estimation Thyroid was carefully dissected out, weighed and kept in 5 *ml* of 20% NaOH for digestion The adrenals, ovaries, uterus and thymus were removed and their wet weights were recorded All the tissues except thyroid were preserved in Bouin's fluid and subjected to histological examination. After fixing, tissue sections were stained with haematoxylin-eosin stain. Thyroid count for radio-activity was taken forty-eight hr after fully digesting it in 5 *ml* of 20% NaOH and then making the volume to 10 *ml* with distilled water. The standard was also diluted with distilled water to a final volume of 10 *ml*. At the time of the injection in the rats, 0.5 μc of I^{131} from the same stock solution was diluted to 5 *ml* with 20% NaOH in duplicate. This served as the standard, for thyroid uptake studies. The thyroid uptake was expressed as percentage of the standard dose administered per *mg* of thyroid weight.

For blood PBI^{131} estimation plasma was separated by centrifugation. Proteins were precipitated out from the plasma by the addition of 15% trichloroacetic acid. The filtrate was washed out four times with trichloroacetic acid and all the precipitate was redissolved in 1 *ml* of 10% NaOH and the volume was made upto 3 *ml*. 0.5 μc of the standard was diluted to 3 *ml* and the percentage of PBI^{131} was calculated, taking the standard counts as hundred per cent *i.e.*

$$\frac{\text{Counts of the plasma}}{\text{Counts of the standard}} \times 100 = \%\text{PBI.}$$

Radio-activity was counted using a well type counter with a 2″ sodium iodide crystal attached to a spectrometer.

RESULTS

Effect of hormones on the body-weight and wet weight of various organs.

TABLE I

Effect of hormone treatment on the body-weight and weights of ovaries, thymus, uterus and adrenals.

Treatment group	*Number of animals*	*Final body-weight/ initial body-weight (g)*	*Mean weight (mg ± S.E.) per 100 g of body - weight*			
			Ovaries	*Thymus*	*Uterus*	*Adrenals*
Olive oil A	6	206±3.76/155±1.4	34.1±3.9	165.5±7.06	134.0±14.8	9.8±1.2
Norethisterone B	6	195±5.9 /178±2.0	31.2±2.01	160.0±13.5	170.0±18.6	7.4±0.85
Ethinyl oestradiol C	6	193±4.03/155±2.3	33.0±3.18	140.0±11.1	189.0±10.2	11.1±0.85
Combination D	6	203±5.2/149±1.36	18.8±1.8	82.0±17.0	180.0±10.5	9.9±2.2
Recovery group E	6	249±7.3/152±2.08	29.6±5.1	130.6±12.2	195.3±41.0	9.2±1.58

Norethisterone : Norethisterone considerably influenced the gain in body-weight of the animals under treatment. The control animals had gained in weight whereas in the norethisterone group, the gain was significantly reduced ($P < 0.05$). There was no change in the mean weights of thyroid, ovaries, thymus and adrenal gland. However, there was an increase in the weight of uterus ($P < 0.05$). (Table I).

Ethinyl oestradiol : Ethinyl oestradiol did not affect the gain in body-weight as compared to the control. There was no significant difference in the weights of thymus, ovaries and adrenals before and after treatment. The uterus showed marked hydroptic changes with a gain in weight which was significant ($P < 0.05$). (Table I).

Effect of ethinyl oestradiol and norethisterone : The combination of the drugs did not influenue the gain in body-weight of the animals. There were definite changes in the weights of sex organs. Thus there was a significant reduction in the weight of ovaries ($P < 0.05$) while the weight of uterus was increased. There was a reduction in the weights of the adrenals and thymus, although the reduction in the weight of either of these organs was not significant ($P > 0.05$). (Table I).

Recovery from the effects of hormones : In order to assess the recovery from the effects produced by the hormones, the hormone therapy was discontinued at the end of seven weeks in group E. There was an increase in the average weights of ovaries, compared with that in group D. The average weight of uterus was not significantly different from that in group D, indicating that the uterus had not recovered from the influence of hormones. Thymus seemed to recover from the influence of the hormones. However, there was no significant difference between the weights of glands of groups D and E. (Table I).

Histological findings : The uterus under the influence of ethinyl oestradiol showed thickening of epithelial lining of endometrium but with norethisterone, the lining was flattened and fewer glands were present. With the combination of hormones, there were fewer glands and in some cases the epithelium showed a tendency towards pseudostratification.

The ovarian tissue of the ethinyl oestradiol treated rats showed maturing follicles. Under the influence of the combined hormone therapy, fewer maturing follicles were seen and no corpora lutea could be detected.

With ethinyl oestradiol or norethisterone the adrenals showed increased cellular activity in the zona glomerulosa. Under the influence of combined hormones, there was a proliferation of zona glomerulosa but both the fasciculata and reticularis zones were reduced making the medulla more prominent.

In the case of thymus, diminution of lymphoid tissue was seen in the group receiving ethinyl oestradiol and combination of the two hormones.

Effect of hormone treatment on thyroid weight, I^{131} uptake and the peripheral PBI^{131}

TABLE II

Effect of hormones on thyroid glands

Treatment group	*No. of animals*	*Mean body weight (g ± S.E.)*	*Mean thyroid weight (mg ± S.E.)*	I^{131} uptake by the gland		*Mean PBI^{131} ±S.E*
				Mean (% of the dose injected) ± S.E	*per mg of thyroid ± S.E.*	
Olive oil A	6	206 ± 3.76	11.0 ± 0.77	47.1 ± 5.4	4.3 ± 0.61	0.46 ± 0.06
Norethisterone B	6	195 ± 5.9	9.8 ± 0.37	47.4 ± 5.6	4.7 ± 0.5	0.57 ± 0.09
Ethinyl oestradiol C	6	193 ± 4.03	10.1 ± 0.54	48.1 ± 3.8	4.9 ± 0.6	0.79 ± 0.029
Combination D	6	203 ± 5.2	12.6 ± 2.59	72.0 ± 7.5	6.3 ± 0.86	0.83 ± 0.08
Recovery group E	6	249 ± 7.3	13.0 ± 1.63	21.9 ± 2.85	1.8 ± 0.25	0.2 ± 0.01

Norethisterone or ethinyl oestradiol or combination of both did not cause any significant change in the average weights of the thyroid glands. There was also no change in the thyroid uptake of I^{131}. However, ethinyl oestradiol treated group and the group given combination therapy showed a significant increase in peripheral PBI^{131} ($P < 0.05$). In the recovery group thyroid weight was unchanged but there was a significant drop ($P < 0.05$) in thyroid I^{131} uptake. Thus the average I^{131} uptake in this group was 1.8% as compared to 4.3% in the control group and 6.3% in group given combination therapy. Further, this reduction in I^{131} uptake paralleled drop in peripheral PBI^{131} level. (Table II).

DISCUSSION

Use of oral contraceptive pills has been accepted as one of the reliable ways in the family planning programme. Since such a pill contains a large dose of oestrogen and some amount of progestagen, it is expected that both these hormones would produce pronounced pharmacodynamic effects on various functions of the body.

There have been various contradictory reports regarding the effect of female sex hormones on thyroid function. Thus it has been reported by Lakshman *et al* (6) that thyroid gland weight is unaltered in female rats receiving norethynodrel. Similarly, administration of Envovid E (combination of norethynodrel and Mestranol) to female monkeys indicated no definite changes in either the weight or histology of the thyroid (5). However, Pincus (7) noted an increase in palpable thyroids in the Puerto Rican study. But Flowers (3) who studied Enovid E in 259 women for 3,510 cycles failed to note an enlargement of thyroids. One of the authors (KDV-unpublished data) noted ten cases of thyroid enlargement during the study of oral contraceptive tablets in 517 Indian women with 5136 cycles. In the present

study, oestrogen or progestagen or combination of both did not produce any significant changes in the thyroid weight inspite of their administration over a period of six weeks.

Neither norethisterone nor ethinyl oestradiol nor the combination (Norlestrin) produced any significant change in I^{131} uptake by thyroid. But ethinyl oestradiol caused significant increase in the peripheral PBI^{131}. Such an effect was seen also with combination and was probably due to oestrogen content of the combination. Stilbestrol has been reported to produce changes in I^{131} uptake by thyroid and pheripheral PBI^{131} (8).

Oestrogens have been reported to produce pronounced increase in thyroxin binding globulin (TBG) (4). However, it should be noted that pheripheral PBI^{131} may be altered by many factors such as

1. Change in the rate of synthesis of thyroid hormone and its turn-over rate from the thyroid.
2. The turnover rate of blood thyroxin by the tissues.
3. The plasma thyroxin binding globulin.

Increased TBG will be reflected in the high PBI^{131} levels. Further, ratio of free to bound thyroxin will decrease and there would be less circulating free hormone. This will in turn release more TSH resulting in thyroid enlargement. However, such an effect was not seen in our study, although there was an increased PBI^{131}. This is evident from the fact that weights of thyroid glands in the control and any of the treated groups did not differ significantly. The period of treatment with hormones in this study was comparatively short.

In our study we have not seen any change in the uptake of radio iodine after the administration of either ethinyl oestradiol or combination of oestrogen and progestagen. It seems therefore, that these hormones do not act primarily on the thyroid gland. The effect on thyroid if any, is likely to be secondary to the changes in the blood PBI : free thyroxin ratio. The most interesting finding was the marked reduction in I^{131} uptake in the recovery group associated with a decrease in PBI^{131}. This can be explained on the basis of reduction in TBG binding capacity because of the withdrawal of oestrogens. This would result in high level of circulating thyroxin which will in turn inhibit TSH and cause a reduction in I^{131} uptake by thyroid. There is also a possibility that after withdrawal of hormones, a new homoestasis is established and that it takes a long time for its return to normal.

SUMMARY

1. The effects of norethisterone, ethinyl oestradiol and combination of the two (Norlestrin) was studied on the various endocrine glands in young female rats.
2. Norethisterone treated animals showed considerable reduction of the gain in body - weight.
3. Both hormones singly or in combination produced definite changes in the weight and in the histology of uterus, ovaries, thymus and adrenals.

4. Norethisterone and ethinyl oestradiol failed to produce any marked changes in the weights of thyroid or I^{131} uptake. The combination produced an increase in weights of thyroid and I^{131} uptake but the results were not statistically significant. However, there was a significant increase in peripheral PBI^{131} in rats treated with the oestrogen and the combination.
5. Withdrawal of oestrogen resulted in significant diminution in PBI^{131} as well as I^{131} uptake by thyroid.

REFERENCES

1. Clark, F. Assessment of thyroid function by the combined use of the serum protein bound iodine and resin uptake I^{131} triiodothyronine. *J. Clin. Endocrine.*, **25** : 39, 1965.
2. Feldman, J.D. Effect of estrogen on thyroid uptake of I^{131} in adrenalectomized rats. *Am. J. Physiol.*, **184** : 369, 1956.
3. Flowers, C.E. Effects on new low dosage form of nor-ethynodrel mestranol. Clinical evaluation and endometrial biopsy study. *J. Am. Med., Assoc.*, **188** : 1115, 1964.
4. Ingbar, S.H. and M.L. Frankel. Regulation of the peripheral metabolism of thyroid hormones. Recent Progress in Hormone Research. 16, 353 as quoted by Eleaner, Mears. In Handbook on Oral Contraception. London. *J. and A. Churchill Ltd.*, 1965.
5. Kar, A.B., H. Chandra, V.P. Kamboj and S.R. Chowdhary. Effect of long term cyclic oral administration of Enovid on genital organs of pre-puberal female Rhesus moneys. *Ind. J. Exp. Biol.*, **3** : 69, 1965.
6. Lakshman, A.B. and W.O. Nelson. Rebound effect of ovulation inhibitory steroids in rats. *Nature*, **199** : 608, 1963.
7. Pincus, G. Suppression of ovulation with reference to oral contraceptive. In Modern Trends in Endocrinology, New York, *H. Gardiner Hill* (Ed.) Ser. 2, Paul B. Heber Inc. 1961, p. 231.
8. Roman, W. and V. Bockner. Paper communicated at the second Asia and Oceania Congress of Endocrinology, Sydney, May, 1963.
9. Soliman, F.A. and E.P. Reineke. Influence of oestrogen and progesterone on radioactive iodine uptake by rat thyroid. *Am. J. Physiol.*, **183** : 63, 1955.