representing thyroid cells. If 89.0 mg of thyroid wet weight contains 470 μg DNA, then 805 μg DNA represents 152.5 mg wet weight when cell size remains constant. Since this is not the case with methimazole treatment, the additional thyroid weight (224.9 mg total minus 152.5 mg due to cell multiplication) is due to increase in cell size. Upon calculating, one finds that 152.5 ÷ 224.9 = 0.68 or that 68% of the increased thyroid gland wet weight is due to cell multiplication (hyperplasia) and that 72.5 ÷ 224.9 = 0.32 or 32% of the increase is due to enlargement of the cells (hypertrophy).

When these calculations are extended to the thyroid glands after 60 days of methimazole, the values are 57% of the increase in weight is due to cell multiplication and 43% is due to increase in cell size. Similarly, the 90 days of methimazole treatment results in a 42% increase in cell multiplication and a 58% increase attributable to an enlargement of the cells.

These observations indicate that cell multiplication is the major cause of the increase in thyroid weight (68%) after goitrogen treatment of 30 days under the stimulus of increased secretion of TSH. After 60 days, cell multiplication accounts for 57% of the increase and after 90 days for 42%.

Histological observations of thyroid glands after goitrogen treatment show the lumen of the follicles free of colloid and the height and presumably the volume of the cells increased. These data indicate that cell size increases markedly with continued goitrogen treatment.

Summary. Mature male fowls were divided into 4 groups of equal TSR. One group served as controls whereas the other 3 groups were injected daily with 0.5 mg tapazole/100 g bw/day for 30, 60 and 90 days. The mean wet thyroid gland weight of the control group was 134.8 mg. After 30 days, the thyroid weight increased 66.8%, after 60 days, the increase was 187.9%, and after 90 days the increased weight was 136.5% above the controls. The determination of the total DNA of each group of thyroid glands indicated the extent of cell multiplication involved and from these data it was possible to estimate the proportion of the weight increase which was due to cell multiplication and the increase due to cell size.

After 30 days the cell multiplication accounted for 68% and cell size for 32% of the increase, after 60 days 57% to cell multiplication and 43% to cell size and after 90 days 42% due to cell multiplication and 58% due to cell size.

1. Hendrich, C. E., Turner, C. W., Proc. Soc. Exp. Biol. & Med., 1964, v117, 218.

2. Webb, J. M., Levy, H. B., J. Biol. Chem., 1955, v213, 107.

3. Richmond, S. B., Statistical Analysis, 2nd Ed., Ronald Press Co., New York, 1964, p186.

4. Kramer, C. Y., Biometrics, 1956, v12, 307.

5. Matovinovic, J., Vickery, Austin L., Endocrinology, 1959, v64, 149.

6. Hendrich, C. E., Turner, C. W., Proc. Soc. Exp. Biol. & Med., 1965, v119, 174.

7. Panda, J. N., Turner, C. W., ibid., 1966, v123, 553.

8. Hayness, R., Glick, B., Poultry Sci., 1960, v39, 1495.

9. Uotila, Unto, Kannas, Osmo, Acta Endocrinol., 1952, v11, 49.

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Endocrine Effects of Pineal Gland and of Melatonin. (32468)

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It has been proposed recently that the pineal gland exerts an inhibitory influence on the gonads and that this inhibition is mediated by a specific neurohumor called melatonin or 5-methoxy-N-acetyl-tryptamine (1). Support for this hypothesis comes from studies which have demonstrated that the weight of the pineal gland(2), and the activ-

ity of hydroxy-indole-O-methyl-transferase (HIOMT) (an enzyme which is essential for the biosynthesis of melatonin and which is found only in the pineal gland(3)) are significantly decreased in situations (for instance, the exposure to constant light) which result in an enhanced activity of the gonads and in a constant estrous syndrome(4).

However, it is not certain whether all the effects exerted by the pineal gland on the reproductive system can be explained by the secretion of melatonin, nor has it been clarified at which level of the hypothalamo-pituitary-gonadal axis pineal gland principles exert their blocking activity; in addition, the study of the endocrine pharmacology of melatonin is still in its preliminary phases. The study reported here was undertaken to clarify some of these points. Experiments were performed both in male and female rats; however, particular emphasis was given to studies in male animals. The reasons are: the literature regarding the effects of pinealectomy and of melatonin administration in female animals is ample and rather uniform in its conclusions (1,5), while that regarding studies in males is scanty and controversial; moreover, in male animals it is easier to evaluate whether pinealectomy and treatment with melatonin influence separately the secretion of the different pituitary gonadotropins (FSH and ICSH-LH), the modifications of the weight of the testes providing a good indication on the amount of FSH being secreted(6) and the changes in the weight of the prostates and of the seminal vesicles being a good index of the release of ICSH-LH(6); in addition, in male animals estrogens are not an interfering factor. It has been reported that pinealectomy increases(7) and pineal extracts decrease(7) hypophyseal weight in female animals; since it has been proved that pituitary weight is influenced by estrogens(8) the effect of pineal principles on this parameter might be due to action on the secretion of estrogens, rather than to a primary effect on the hypophysis.

Materials and methods. Sexually mature male rats (initial body weight 245 ± 8 g) were pinealectomized following the technique of Kitay and Altschule(9). Sham-operated animals were used as controls. Both pinealectomized and sham-operated animals were killed 12 days following the operation.

Melatonin was injected subcutaneously for 21 days into prepuberal male and female animals (daily dose: 200 μg/rat). Treatments were initiated when females were 22 days old

TABLE I. Effect of Pinealectomy on Weights of Endocrine Structures in Male Rats.

Groups*	Final body wt, g	Pituitary wt, mg	Testes wt, g	Seminal vesicles wt, mg	Prostates wt, mg
Sham-operated (20)	263.7 ± 7.2	7.7 ± .34	2.82 ± .21	290.5 ± 11.3	184.8 ± 15.2
Pinealectomized (15)	258.3 ± 5.4	8.1 ± .49	3.24 ± .19	530.7 ± 18.1†	274.1 ± 17.0†

Values are Means ± SE
* No. of rats in parentheses.
† $P \leq .001$

TABLE II. Effect of Systemic Administration of Melatonin on Weights of Endocrine Structures in Male Rats.

Groups*		Body wt, g	Pituitary wt, mg	Testes wt, g	Seminal vesicles wt, mg	Prostates wt, mg
Controls (12)	initial	96 ± 4				
	final	223 ± 8	7.3 ± .50	2.53 ± .29	240.3 ± 10.6	167 ± 11.0
Melatonin (12)						
200 μg/rat	initial	97 ± 4				
s.c.	final	227 ± 7	6.9 ± .42	2.69 ± .31	190.1 ± 15.0†	134 ± 10.0†

Values are Means ± SE.
* No. of rats in parentheses.
† $P \leq .02$

and males 30 days old. Controls were similarly treated with saline. All animals were killed 24 hours after the last injection.

Results and discussion. Pinealectomy performed in sexually mature male rats does not modify pituitary weight. Testicular weight is enhanced, although not significantly. A very significant increase in the weights of the prostates and of the seminal vesicles is obtained. No relevant changes in body weight are observed following the operation (Table I).

Treatment of 30-day-old male rats (for 3 weeks) with a rather high dose of melatonin results in the following changes in endocrine structure, the weights of the pituitary glands and of the testes are unmodified; the prostates and the seminal vesicles are significantly atrophied (Table II).

In prepuberal female rats (22 days old), administration of melatonin for 3 weeks results in a significant retardation of the canalization of the vagina. This indicates that puberty has been retarded by the treatment. Pituitary weight is significantly decreased following treatment, as are the weights of the ovaries and of the uteri (Table III). There was a small reduction in body growth in female animals treated with melatonin. This effect was not observed in males.

The increase in weight of the testes and of the testosterone-dependent structures (prostates and seminal vesicles) following pinealectomy clearly indicates that the ablation of this gland induces simultaneously an increase of the secretion of FSH and ICSH-LH. This would be consistent with the hypothesis that the inhibitory action exerted by the pineal gland on the gonads is not due to a peripheral effect, but rather to a block of gonadotropin secretion; an effect of the pineal gland on gonadotropin secretion is supported also by data from our laboratory which indicate that pinealectomy in mature male rats is followed by a dramatic increase in pituitary LH(10) and FSH stores (Mess, unpublished observations). The inhibition of gonadotropin secretion might take place either directly at the pituitary level or on the neural structures involved in the control of pituitary function; this second hypothesis seems more probable, since other studies performed in this laboratory have indicated that brain implants of pineal tissue significantly reduce LH secretion(10).

Melatonin, even when used in rather large doses, does not counteract completely the effects of the removal of the pineal gland. Melatonin significantly reduces the secretion of ICSH-LH (as indicated by the reduced weights of prostates and seminal vesicles), but apparently does not modify the secretion of FSH (as indicated by the normality of testes weight). Pinealectomy does not modify pituitary weight in male rats; this result indicates that the increase in pituitary weight in pinealectomized females, reported by previous authors(7), is not a primary effect of the ablation of the gland, but probably the consequence of the increased estrogen levels which accompany the constant estrus situation induced by the operation. This is confirmed by the observation that melatonin reduces pituitary weight only in female rats. That estrogen secretion is reduced following melatonin administration is indicated also by the reduced weights of

TABLE III. Effect of Systemic Administration of Melatonin on Puberty and on Weights of Endocrine Structures in Female Rats.

Groups*		Body wt, g	Pituitary wt, mg	Ovary wt, mg	Uterus wt, mg	Vaginal opening (days)
Controls (12)	initial	63 ± 3				
	final	141 ± 6	6.1 ± .42	39.5 ± 4.1	185.2 ± 15.7	37.1 ± 1.3
Melatonin (12)						
200 μg/rat	initial	64 ± 3				
s.c.	final	134 ± 5	4 6 ± .18‡	28.6 ± 2.5†	100.5 ± 39.7‡	42.2 ± .8†

Values are Means ± SE.
* No. of rats in parentheses.
† $P \leq .01$
‡ $P \leq .001$

the uteri; all these data can be explained as being due to an inhibitory effect of melatonin on LH secretion.

The demonstration that melatonin does not counteract all the effects of pinealectomy, and in particular that the compound does not reduce FSH secretion, suggests that the pineal gland inhibits FSH secretion through a mechanism independent from melatonin. Although the hypothesis of direct neural influences cannot be disregarded, it appears more logical that pineal inhibition of FSH secretion is humoral in nature (through the secretion of other indole compounds, like hydroxy-tryptophol and 5-methoxy-tryptophol, or of proteins and peptides(11,12)).

The suggestion proposed here, that the pineal gland controls the secretion of both FSH and LH, while melatonin reduces only the secretion of LH, is supported by a few data obtained in other laboratories. There is general agreement that pinealectomy results in enlarged seminal vesicles and ventral prostrates(13,14); testicular hypertrophy following pinealectomy has also been reported (13,15,16,17), although others have denied it(14). A role of the pineal gland in controlling the secretion of FSH is also supported by the observation that pinealectomized hamsters do not undergo as great or as rapid testicular atrophy in winter(18) or following exposure to constant dark periods or removal of both eyes(19) as do normal controls. In addition, pinealectomy enhances testicular compensatory hypertrophy following unilateral castration(20). That melatonin reduces the weight of testosterone-dependent structures, leaving unmodified FSH-dependent phenomena, like spermatogenesis(21) and testicular weight(22,23,24), has also been shown before; on the other hand, crude pineal extracts have been shown to inhibit the release of FSH both *in vitro*(22) and *in vivo*(25).

The experiments in which it was shown that melatonin retards puberty in female animals and reduces ovarian and uterine weights are confirmatory of those of Wurtman(see 1 for references), who found that all these effects can be obtained with smaller doses of melatonin than we used. This indicates that LH secretion is more sensitive to the effect of melatonin than might appear from our results.

It has not been attempted to explain why chronic treatment with melatonin results in retarded vaginal opening. The demonstration presented in this and in another paper(10), that the primary mode of action of melatonin is the suppression of the secretion of LH (and consequently of estrogens), provides an explanation for this effect. It has been reported that the onset of puberty is accompanied by significant changes in the secretion of pituitary LH and of the hypothalamic LH-Releasing Factor (LH-RF)(26); exogenous estrogens have been shown to advance puberty either when given systemically (27) or when implanted in the median eminence region(28) and to advance prepuberal modifications of synthesis and release of LH and LH-RF(27). It is possible that melatonin stops all these events because it suppresses, as shown by the experiments reported here, the estrogen-LH interplay which is essential for initiating puberty. This interpretation is supported by the data of Adams *et al*(29), which indicate that melatonin, administered before puberty, inhibits the drop of pituitary LH which is usually observed at the time of vaginal canalization.

Summary. 1. Pinealectomy when performed in male rats results in a significant increase in weight of the testes, the prostates and the seminal vesicles. This suggests that the pineal gland usually inhibits the secretion of LH and of FSH. 2. Melatonin administered to male rats diminishes the weights of prostates and of seminal vesicles, but does not change testicular weight, indicating that exogenous melatonin suppresses the secretion of LH, but does not interfere with FSH release. 3. In prepuberal female rats melatonin retards puberty and decreases pituitary weight as well as the weights of the ovaries and the uteri, confirming that melatonin reduces LH secretion. 4. It is suggested that the pineal gland exerts an anti-FSH effect through compounds different from melatonin.

1. Wurtman, R. J., Neuroendocrinology, Academic Press, New York, 1967, v2, 19.

2. Fiske, V. M., Pound, J., Putman, J., Endo-

crinology, 1962, v71, 130.
3. Wurtman, R. J., Axelrod, J., Phillips, L. S., Science, 1963, v142, 1071.
4. Wurtman, R. J., Axelrod, J., Chu, E. W., ibid., 1963, v141, 277.
5. Kitay, J. I., Neuroendocrinology, Academic Press, 1967, v2, 641.
6. Davidson, J. M., ibid., 1966, v1, 565.
7. Wurtman, R. J., Altschule, M. D., Holmgren, U., Am. J. Physiol., 1959, v197, 108.
8. Palka, Y. S., Ramirez, V. D., Sawyer, C. H., Endocrinology, 1966, v78, 487.
9. Kitay, J. I., Altschule, M. D., The Pineal Gland, Cambridge, 1954.
10. Fraschini, F., Mess, B., Martini, L., Endocrinology, 1967, in the press.
11. McIsaac, W. M., Taborsky, R. G., Farrel, G., Science, 1964, v145, 63.
12. Ebels, I., Moszkowska, A., Scemama, A., C. R. Acad. Sci., Paris, 1965, v260, 5126.
13. Kincl, F. A., Benagiano, G., Acta Endocrinol., 1967, v54, 189.
14. Roth, W. D., Am. Zool., 1964, v4, 53.
15. Izawa, Y., J. Physiol., 1926, v77, 126.
16. Carnicelli, A., Saba, P., Cella, P. L., Marescotti, V., Folia Endocrinol., Pisa, 1963, v16, 229.
17. Houssay, A. B., Pazo, J. H., Abst. of III International Pharmacological Congress, 1966, Sao Paulo, p147.
18. Cziba, J. C., Girod, C., Durand, N., C. R. Soc. Biol., Paris, 1964, v158, 742.
19. Hoffman, R. A., Reiter, R. J., Science, 1965, v148, 1609.
20. ———, Nature, 1965, v207, 658.
21. Kappers, J. A., Am. Zool., 1964, v4, 47.
22. Moszkowska, A., Rev. Suisse Zool., 1965, v72, 145.
23. Ebels, I., Prop, N., Acta Endocrinol., 1965, v49, 567.
24. Tilstra, B., Prop, N., Acta Morph. neerl-scand., 1962, v5, 289.
25. Thieblot, L., Blaise, S., Ann. Endocrinol., Paris, 1963, v24, 270.
26. Ramirez, V. D., Sawyer, C. H., Endocrinology, 1966, v78, 958.
27. ———, ibid., 1965, v76, 1158.
28. Martini, L., Fraschini, F., Mess, B., Motta, M., Path. Biol., Paris, 1967, v15, 186.
29. Adams, W. C., Wan, L., Sohler, A. J., Endocrinol., 1965, v31, 295.

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Depression of Reticuloendothelial Phagocytic Activity by Phytohemagglutinin. (32469)

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Phytohemagglutinin (PHA) is an extract of the red kidney bean *Phaseolus vulgaris* which effects a remarkable mitogenic action on leukocytes *in vitro*(1). PHA also causes the agglutination of human erythrocytes as well as those from several animal species(2). Many studies have been conducted on the *in vitro* action of PHA on lymphocytes. (Recently reviewed in reference 1.). In contrast, only a limited number of studies have been performed in humans and in laboratory animals. Conflicting reports have appeared about the effect of PHA in the treatment of human hypoplastic or aplastic anemias(3,4). While PHA has been shown to stimulate the mitotic activity of bone marrow cells in young rats (5), the injection of PHA did not produce significant changes in the hematocrit, total white blood cell count, serum globulins or in the histologic pattern of the lymphoreticular system(6). The administration of PHA suppresses antibody production in rats(7) when given in appropriate dosages and at a specified time before exposure to an antigen.

It has been reported that the changes which develop in mouse spleen cells after the *in vivo* administration of PHA(8) resemble those obtained following the addition of PHA to cells in tissue culture(1). It seemed reasonable, therefore, to assume that similar alterations could occur in other reticuloendothelial cells which might lead to an alteration of their function. The present study was undertaken to test this hypothesis and thus to determine the effect of PHA on reticuloendothelial system (RES) phagocytic function.

Materials and methods. The animals used