already been reduced by about 8 hours from that originally required, and a further decrease is anticipated by using higher-pressure systems and optimizing operating parameters such as temperature and elution gradient.

The capability of the system we have described is limited to the analysis of ultraviolet-absorbing constituents of urine; however, it could be extended by incorporation of additional detection systems. For example, if the ninhydrin colorimetric detection system(13) and a carbohydrate colorimetric technique recently developed by Green(14) could be integrated into the present analyzer (while using the same separation system), the resulting instrument would be able to detect a much larger number of compounds.

1. Altman, P. L., Dittmer, D. S., Blood and Other Body Fluids, Fed. Am. Soc. Exp. Biol., Washington, D.C., 1961.

2. ———, Biology Data Book, ibid., 1964.

3. Damm, H. C., ed., Handbook of Clinical Laboratory Data, Chemical Rubber Co., Cleveland, 1965.

4. Long, C., ed., Biochemists' Handbook, E. & F. N. Spon, Ltd., London, 1961.

5. Gutman, A. B., Yu, T. F., Am. J. Med., 1957, v23, 699.

6. Adams, W. S., Davis, F., Nakantani, M., ibid., 1960, v28, 726.

7. Kerr, J. W., Lancet, 1964, 1963-II. 709.

8. Eiduson, S., Geller, E., Yuwiler, A., Eiduson, B. T., Biochemistry and Behavior, D. Van Nostrand Co., Inc., New York, 1964.

9. Anderson, N. G., Science, 1966, v154, 103.

10. Anderson, N. G., Green, J. G., Barber, M. L., Ladd, Sr. F. C., Anal. Biochem., 1963, v6, 153.

11. Cohn, W. E., J. Biol. Chem., 1960, v235, 1490.

12. Reid, J. C., Wong, E. C., Applied Spectroscopy, 1966, v20, 320.

13. Moore, S., Stein, W. H., J. Biol. Chem., 1954, v211, 893.

14. Green, J. G., Nat. Cancer Inst. Monogr. 1966, No. 21, 447.

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Effect of Melatonin on Thyroid Hormone Secretion Rate and Feed Consumption of Female Rats.* (32044)

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The physiological role of the pineal gland has long been a mystery, but numerous reports have appeared during the last ten years regarding the status of this gland. According to Wurtman *et al*(1) the pineal is an endocrine gland and secretes the hormone melatonin which affects the reproductive system, primarily in females. Pinealectomy in the immature rat produced ovarian hypertrophy (2,3) while administration of pineal extracts caused ovarian atrophy(3,4). It was then shown by Wurtman *et al*(5) that the effects produced by pinealectomy were similar to those produced by exposure to constant light, but these were not additive. The ability of the rat pineal gland to synthesize melatonin was reported to be markedly reduced by exposure to light(6) and enhanced by constant darkness(7,8). It would thus appear that melatonin depressed the precocious development of the ovary; and light, by depressing melatonin secretion, stimulates the gonadotrophic hormones and ovarian development.

The possible relation of the pineal gland and melatonin to other endocrine gland functions requires study. Evidence indicating a relation to thyroid gland weight(9), adrenal gland(8,10,11,12,13,14) and the pituitary gland(3,15) has been presented. Kitay and Altschule(16) summarized the literature on the relation of pinealectomy to the thyroid gland citing references indicating hypertrophy or no change. Malm *et al*(17) reported that pinealectomy stimulated the growth of rats

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up to the 14th week. Ishibashi *et al*(18) pinealectomized immature rats and noted a 5.77% increase in feed consumption and a 11.8% increase in thyroid hormone secretion rate (TSR). Injection of 40 μg/day of melatonin depressed feed consumption 12%; whereas, 20 μg/day depressed TSR 16%.

In a continuation of our studies on the effect of various hormones and physiological factors on the thyroid hormone secretion rate (TSR), and feed consumption, the present investigations were undertaken to study the effect of increasing doses of melatonin on TSR of female rats of increasing age and on feed intake and endocrine gland weight at the end of the experiment.

Materials and methods. The thyroid hormone secretion rate (TSR) of 57 female rats of the Sprague-Dawley-Rolfsmeyer strain (25 days of age, with an average weight of 55 g) was determined by the method described previously by Grosvenor and Turner (19). The TSR, as estimated by this procedure, will be slightly higher than the actual TSR of the animal, because the final level of thyroxine which blocks the release of thyroidal I^{131} must be in slight excess of the animal's own secretion of the thyroid hormones. Secondly, as the level of thyroxine is increased by 0.25 μg/100 g bw every 48 hours, and the dose previous to the final one does not completely block the release of thyroid I^{131}, the error must be less than 0.25 μg/100 g bw. The rats were then divided into 3 subgroups, each having the same mean TSR. Groups I and II, of 15 rats each, were controls, Group I being untreated while Group II was injected s/c with 0.5 ml of normal saline/day from a week before the start of TSR, until the TSR was estimated. Rats of Group III received s/c 50 μg dose of melatonin/day dissolved in 0.5 ml of physiological saline/rat, and this treatment was continued from a week before the start of TSR estimation until the TSR of that animal was estimated. TSR estimation of the 3 groups with treatments as outlined above was started when the rats were 55 days of age. TSR of the 3 groups was again estimated at monthly intervals, starting at the age of 85 days and 115 days respectively, after a similar treatment of various groups as of 55 days of age, except that the dose of melatonin was increased to 75 μg/rat at 85 days and 100 μg/rat when they were 115 days of age at the start of TSR determination.

Twenty-four rats, 12 each from Groups II and III above, were housed in individual metabolism cages for determination of their feed consumption, for a period of 12 days, when they were 115 days old. Group II was injected s/c with 0.5 ml of physiological saline/rat/day during the period of determination of feed consumption.

Group III was injected s/c with 100 μg of melatonin in 0.5 ml physiological saline/rat/day during a 12-day period of feed consumption determination.

Three days were allowed for adjustment to feeding and watering conditions in individual rodent metabolism cages so constructed as to prevent coprophagy and feed wastage before the start of food consumption measurements.‡ A weighed quantity of an excess of ground Purina Lab Chow was provided for each rat and the residue weighed back, every other day. Thus, feed consumption every 2 days was measured for 12 consecutive days and an average/day was estimated/rat as well as/100 g bw. A record of the body weights of the individual rats was maintained in addition to the feed consumed by each rat. Average weight of each rat for the 12-day period was taken into account in calculating the feed consumption/day/100 g bw. Drinking water was supplied to rats *ad libitum.*

At the end of the final TSR estimation which was started when the rats were 115 days of age, the 3 groups were killed, their pituitaries, ovaries and the adrenals were removed and immediately weighed.

Results. The mean TSR of Groups I, II and III were respectively 1.21 μg L-T_4/100 g bw, 1.23 μg/100 g bw, and 0.95 μg/100 g bw, when the estimation was started in rats at 55 days of age, thus showing a highly significant ($P<.025$) reduction of 22.8% in TSR with a 50 μg dose of melatonin given a week before

‡ Manufacted by Acme Metal Products, Inc., Chicago.

TABLE I. Effect of Melatonin on TSR of Female Rats of Increasing Age.

Group	No. of surviving rats	Age (days)	Mean body wt (g)	Mean TSR/100 g bw (μg ± S.E.)	Reduction in TSR (%)
I—Control	14	55	166	1.21 ± .08[a]	
II—Control (.5 ml saline)	15	55	166	1.23 ± .11[b]	
III—50 μg melatonin in .5 ml saline	26	55	163	.95 ± .07[c]	22.8
I—Control	14	85	203	1.12 ± .10	
II—Control (.5 ml saline)	15	85	203	1.08 ± .05[d]	
III—75 μg melatonin in .5 ml saline	25	85	212	.92 ± .04[e]	14.8
I—Control	14	115	228	.88 ± .10	
II—Control (.5 ml saline)	13	115	218	.88 ± .13	
III—100 μg melatonin in .5 ml saline	23	115	231	.80 ± .07	9.1

S.E. = Standard error of mean.
Student "t" probability: a *vs* c, $P < .025$; b *vs* c, $P < .025$; d *vs* e, $P < .01$.

and during the TSR estimation (Table I).

TSR of the same groups of rats when started at the age of 85 days was respectively 1.12 μg L-T_4/100 g bw, 1.08 μg L-T/100 g bw and 0.92 μg L-T_4/100 g bw for Groups I, II and III thus showing a highly significant ($P < .01$) reduction of 14.8% in the melatonin-treated rats over those injected s/c with the same volume of physiological saline. The dose of melatonin was 75 μg/rat at this age (Table I).

When started at the age of 115 days, the respective TSR of the Groups I, II and III was 0.88 μg L-T_4/100 g bw, 0.88 μg L-T_4/100 g bw and 0.80 μg L-T_4/100 g bw. The dose of melatonin at this age was increased to 100 μg/rat, which showed a non-significant reduction of 9.1% in TSR (Table I).

A non-significant reduction of 8.76% in feed consumption of the melatonin-treated rats (Group III) was observed, based on the average of 12 days feed consumption/rat/100 g bw (Table II).

The ovary and the pituitary weights of the melatonin-treated (Group III) rats showed a highly significant reduction of 30.1% ($P < .005$) and 34.6% ($P < .001$) respectively, per 100 g bw as compared to those injected s/c with the same volume of physiological saline (Group II) for the same period. The adrenals, however, showed a highly significant increase of 22.7% ($P < .005$) per 100 g bw over those of the control group (Table III).

Discussion. There is sufficient evidence in the literature to indicate that melatonin satisfies the criteria of a hormone(1). In addition to the effect of pineal extracts and melatonin on gonad function, especially in young immature female rats, the thyroid and the adrenals have been thought to be in some way influenced by the pineal(16). They suggested also that the effect of pineal extracts on the gonads might be mediated by the adenohypophysis. Injection of pineal extracts were reported to produce histologic changes in the thyroid gland, suggestive of depression of thyroid function(20). Baschieri *et al*(9) found that the thyroid weight in animals treated with 150 μg of melatonin every day

TABLE II. Effect of Melatonin on Feed Consumption of Female Rats (at End of Experiment).

Group	No. of rats	Avg body wt for 12-day period (g)	Avg daily feed consumption/rat ± S.E. (g)	Range of feed consumption/rat (g)	Avg daily feed consumption /100 g body wt (g)	Reduction in feed consumption/100 g body wt
II Normal saline	12	218.50	13.22 ± .48	11.52–16.04	6.05 ± .5	
III Melatonin, 100 μg	12	232.4	12.82 ± .28	11.11–14.35	5.52 ± .3	8.76%

TABLE III. Effect of Melatonin on Adrenal, Pituitary and Ovary Weights in the Female Rats.

	Group I	Group II	Group III	Change due to melatonin (%)	Significance level
No. of surviving rats	14	13	23		
Avg body wt	227 g	219 g	230 g		
Ovarian wt—					
Per rat	82.6 mg ± 3.4	83.7 mg ± 2.6	61.4 mg ± 2.1	−26.64	P <.001
Per 100 g bw	36.4 " ± 1.7	38.2 " ± 3.2	26.7 " ± .09	−30.1	P <.005
Adrenal wt—					
Per rat	35.7 " ± 1.6	36.7 " ± 1.8	47.2 " ± 2.1	+28.6	P <.001
Per 100 g bw	15.7 " ± .8	16.7 " ± .8	20.5 " ± .9	+22.8	P <.005
Pituitary wt—					
Per rat	11.1 " ± .5	11.4 " ± .5	7.8 " ± .2	−31.6	P <.001
Per 100 g bw	4.9 " ± .2	5.2 " ± .2	3.4 " ± .11	−34.6	P <.001

for 10 days was markedly lower (23.5%) than that of the control group. The present investigation is in full conformity with the above findings and establishes a quantitative effect of melatonin on TSR. The results further indicate that the greatest effect of melatonin on TSR is in the immature rats of 55 days of age (22.8% reduction in TSR), compared to the older rats of 85 days of age (14.8% reduction in TSR) or 115 days of age (9.1% reduction in TSR) even though the dose was increased from 50 μg to 75 μg and 100 μg/day/rat, respectively on the 85th and 115th day of age.

In the previous study (18) it was shown that pinealectomy of immature rats increased feed consumption 5.8% whereas 40 μg/day depressed feed consumption 12%. The nonsignificant reduction of 8.76% in the feed consumption at the end of the experiment may be due to the reduced effectiveness of melatonin with increasing age.

A marked reduction in ovarian weight induced by the administration of a pineal extract in the immature rat was reported by Kitay and Altschule(4), Wurtman *et al*(3,1) and Adams *et al*(15). Wurtman *et al*(3) reported also that pineal extracts produced a decrease in pituitary weight and larger doses caused a significant reduction in adrenal weight. The pituitaries of melatonin-treated rats were smaller and contained a higher concentration of luteinizing hormone(15). Farrell(10) reported that extracts of cattle pineal glands stimulated aldosterone secretion in decerebrated dogs without affecting the secretion of cortisol. The rats in the present experiment were sacrificed at about 130 days of age. The increase in adrenal weight observed, in contrast to the observations of Wurtman *et al*(3), may be due to the maturity of the animals.

Summary. TSR estimation of Sprague-Dawley-Rolfsmeyer strains of rats at 55 and 85 days of age was found to be highly significantly reduced by 22.8% and 14.8% respectively with subcutaneous injection of 50 μg melatonin/rat at 55 days, and 75 μg/rat at 85 days but not by 100 μg/rat at 115 days of age. These results indicate that melatonin depresses TSR but the effect appears to be reduced with advaancing age. Feed consumption was reduced 8.76%, a nonsignificant reduction, in 115-day-old rats injected s/c with 100 μg melatonin/day/rat for a period of 12 days. The effect may be cumulative due to increasing doses of melatonin during each TSR estimation at monthly intervals. At the end of the experiment, the mean ovarian weight showed a highly significant reduction of 30.1% and the pituitary weight of 34.6% in comparison with the control group. In contrast, the mean adrenal weight showed a highly significant increase of 22.7%.

1. Wurtman, R. J., Axelrod, J., Chu, E. W., Science, 1963, v141, 277.

2. Kitay, J. I., Endocrinology, 1954, v54, 114.

3. Wurtman, R. J., Altschule, M. D., Holmgren, U., Am. J. Physiol., 1959, v197, 108.

4. Kitay, J. I., Altschule, M. D., Endocrinology, 1954, v55, 782.
5. Wurtman, R. J., Altschule, M. D., Wurtman, J. I., Acta Endocrinol., 1961, v36, 617.
6. Wurtman, R. J., Axelrod, J., Chu, E. W., Fischer, J. E., Endocrinology, 1964, v75, 266.
7. Wurtman, R. J., Axelrod, J., Phillips, L. S., Science, 1963, v142, 1071.
8. Wurtman, R. J., Axelrod, J., Fed. Proc., 1964, v23, 206.
9. Baschieri, L., Deluca, F., Cramarossa, L., Demartino, C., Oliverio, A., Negri, M., Experientia, 1963, v19, 15.
10. Farrell, G., Fed. Proc., 1960, v19, 601.
11. Farrell, G., McIsaac, W. M., Arch. Biochem., 1961, v94, 143.
12. Everitt, A. V., Huang, C. Y., Australian J. Sci., 1962, v25, 115.
13. Kennedy, A., Kilshaw, A., Reid, N. C. R. W., Taylor, W. H., Brit. Med. J., 1962, No. 5305, 641.
14. Palkovits, M., Foldvari, P. I., Endocrinology, 1963, v72, 28.
15. Adams, W. C., Wan, Lillian, Sohler, A., J. Endocrinol., 1965, v31, 295.
16. Kitay, J. I., Altschule, M. D., The Pineal Gland, Harvard Univ. Press, Cambridge, Mass., 1954.
17. Malm, O. J., Skaug, O. E., Jaerde, P. L., Acta Endocrinol., 1959, v30, 22.
18. Ishibashi, T., Hahn, D. W., Srivastava, L., Kumaresan, P., Turner, C. W., Proc. Soc. Exp. Biol. & Med., 1966, v122, 644.
19. Grosvenor, C. E., Turner, C. W., ibid., 1958, v99, 517.
20. Miline, R., Scepovic, M., Ann. D'Endocrinol., 1959, v20, 511.

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Studies with Clearing Factor V. State of Tissue Lipases After Injection of Heparin.* (32045)

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Lipoprotein lipase (Clearing Factor) is a lipolytic enzyme (EC 3. 1. 1. 3.) which appears in the plasma after intravenous injection of heparin. The presence in the normal rat heart of an enzyme identical with Clearing Factor has been demonstrated(1). It was shown that the increase in the Clearing Factor concentration of plasma after heparin injection was correlated with the depletion of this enzyme in the heart tissue(1).

Lipolytic activity similar to lipoprotein lipase was also reported in kidney(2), liver (3), lung(4), adipose tissue(5) and lactating mammary gland(13). In the plasma the presence of an endogenous lipoprotein lipase was reported in rats(7) and in a small percentage of people(12); a heat stable monoglyceridase(8) was also reported. A hormone sensitive β-monoglyceridase in adipose tissue was reported by Steinberg *et al*(6). The state of the tissue lipases after heparin injection, however, has not been investigated.

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The work presented in this paper demonstrates the relationship of the tissue lipases to the plasma Clearing Factor that appears after injection of heparin. Evidence is also presented that all tissue lipases and lipoprotein lipases are the source of the postheparin plasma Clearing Factor enzymes. Particle bound β-monoglyceridase of adipose tissue is demonstrated to be markedly affected by heparin injections.

Methods and materials. Rat tissues were prepared from Holtzman rats (150-250 g) which were killed by etherization. Five rats were sacrificed for each assay and the tissues were washed thoroughly with cold physiological saline to remove the blood. Pooled tissues were homogenized in a Waring blendor for 3 minutes with 150 ml of acetone at −15°C. The contents were vacuum filtered and acetone powders were stored under vacuum at −15°C.

The enzyme was prepared by extracting tissue acetone powders (50 mg per ml in the final incubation mixture) with 0.25 M am-