

Fish that received both Methallibure and thiourea showed degranulation of both types of basophil in the PPD. The ovaries from these fish showed atresia and blockage of vitellogenesis. Thyroid follicle cells appeared to show a somewhat less marked loss of colloid than in fish treated with thiourea alone.

On the basis of these results it seems reasonable to suggest that the small central Type I basophils are thyrotropes and the larger ventral Type II cells are gonadotropes. Methallibure appears to act by inhibiting the production of pituitary gonadotropin(s) and reducing the production of TSH.

Preliminary results for another teleost, *Hypseleotris galii*¹⁶, suggest a similar distribution of these cell types. The work reported here is part of a study of the reproductive physiology of several teleost species using Methallibure treatment to replace the classical technique of surgical hypophysectomy¹⁷.

Zusammenfassung. Die versuchsweise Identifizierung gonadotroper Zellen der Teleostier-Hypophyse (*Plecto-plites ambiguus*) gelang nach Behandlung mit Methallibure (I.C.I. 33, 828) und Thiourea.

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¹⁶ N. J. MACKAY, in preparation.
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Pineal Weight Response to a Dietary Variable in *Microtus montanus*

Several investigators have demonstrated a correlation between changes in the pineal gland and the reproductive system relative to photoperiod. The laboratory rat maintained under constant dark has a decreased incidence of estrus associated with an increase in pineal weight and hydroxy-indole-*O*-methyl (HIOMT) transferase activity¹. In constant light, estrus is prolonged, ovarian hypertrophy is observed and pineal weight and HIOMT activity are decreased². HOFFMAN and REITER³ demonstrated in hamsters that gonadal atrophy which occurs as a result of blinding or artificial short days (1 h of light) is prevented in pinealectomized animals.

It has been established for a number of different species that long photoperiod is highly stimulatory to reproduction while short photoperiod has no effect or is a negative stimulus. The montane meadow vole, *Microtus montanus*, a strict herbivore, shows a positive reproductive response to long photoperiod⁴. Furthermore, NEGUS and PINTER⁵ have demonstrated a positive reproductive response to supplements of fresh green plants in the diet of this species.

The present study was undertaken to determine whether or not changes in photoperiod or addition of fresh green plants to the diet could affect weight changes in the pineal gland of *M. montanus*. Most of the animals used in this study were young subadults of uniform age and size, obtained from an outbred colony of *M. montanus* maintained in our laboratory. One group of experimental animals was obtained directly from wild populations at

the Jackson Hole Biological Research Station, Wyoming. This group was taken into captivity during late October, 1968, when the entire population was in a non-breeding condition. From time of capture and throughout the experimental period, these animals were maintained under short photoperiod (8L, 16D).

Animals were caged either in pairs or singly and were given Purina rabbit chow and water ad libitum. The outer leaves of head lettuce were fed as a dietary supplement to experimental animals. Daily artificial illumination provided by cool white fluorescent lights (250 ft candles) was controlled by a 24-h clock-switch. Temperature was maintained at 70–72°F.

Generally, the laboratory animals were maintained under a particular light regime for 21 days prior to a succeeding 21 days of experimental diet administration. At the termination of each experiment, animals were sacrificed in the middle of the dark period (Table I). The wild caught animals were maintained under 8 h

¹ R. J. WURTMAN, J. AXELROD and L. S. PHILLIPS, *Science* 142, 1071 (1963).
² R. J. WURTMAN, J. AXELROD, E. W. CHU and J. E. FISCHER, *Endocrinology* 75, 266 (1964).
³ R. A. HOFFMAN and R. J. REITER, *Life Sci.* 5, 1147 (1966).
⁴ A. J. PINTER and N. C. NEGUS, *Am. J. Physiol.* 208, 633 (1965).
⁵ N. C. NEGUS and A. J. PINTER, *J. Mammal.* 47, 596 (1966).

Table I. Effect of daily dietary supplement of fresh plant substances on pineal weight in *Microtus montanus*

Group	No. of animals	Sex	Light regime	Treatment	\bar{x} Pineal weight (mg)	Level of significance (P)
1	9	♀	8L 16D	Greens	0.096 ± 0.047*	0.025
	9	♀	8L 16D	No greens	0.152 ± 0.057	
2	18	♂	8L 16D	Greens	0.096 ± 0.028	0.025
	16	♂	8L 16D	No greens	0.123 ± 0.043	
3	16	♂	12L 12D	Greens	0.091 ± 0.040	0.025
	19	♂	12L 12D	No greens	0.130 ± 0.068	
4	27	♂	16L 8D	Greens	0.086 ± 0.040	0.01
	24	♂	16L 8D	No greens	0.116 ± 0.047	

* Standard Deviation.

light/16 h dark for 3 weeks. Half of the animals were fed the experimental diet for the entire 3-week period. These animals were killed in the middle of the light period (Table II). Pineal weights were obtained by weighing freshly fixed pineals on a Cahn electrobalance (sensitivity 0.1 μ g). After fixation in AFA, reproductive tracts and gonads were weighed on a Mettler H-16 balance (sensitivity 0.1 mg).

In all cases those animals receiving a daily dietary supplement of fresh, green lettuce leaves had significantly lower pineal weights than those animals receiving only the laboratory feed (Table I). As all the laboratory animals were subadults in breeding condition at the start of the various regimes, the experimental diet would not be expected to elicit a weight change in the reproductive organs. In fact, no such response was observed.

The pineal weight and reproductive organ weight responses of wild *M. montanus* are summarized in Table II. While weights of the greens fed male gonads and pineals are significantly different from those of the no greens males (p 0.05), those of the females are not. However, weight changes in females do exhibit a trend consistent with the observed results. The wild animals were not in breeding condition and did not represent a uniform group with respect to age or size.

The weights of the pineals from no greens animals are not significantly different under the various light regimes. There is a trend toward heavier pineals under longer dark periods, however. The no greens pineals from the wild *M. montanus* are significantly lighter than those of the laboratory animals. The wild animals were sacrificed in the middle of the light period as compared to the

laboratory animals which were sacrificed in the middle of the dark period. Finally, the pineal weights of the animals receiving the experimental diet were all similar. This suggests that a maximum response to the green plant supplement was achieved regardless of the length of photoperiod.

Dietary supplements of fresh green plant food have been shown to stimulate reproductive responses in *M. montanus*⁶. From the results presented here, a dietary supplement of green plant food is again correlated with reduced pineal weight. Some preliminary investigations on the effects of diet on HIOMT activity in the pineal indicate that animals receiving a green plant supplement show a 25% reduction in HIOMT activity as compared to control animals⁶. Other workers have shown that HIOMT activity in the pineal is reduced during periods of light⁷.

With increasing frequency, investigators are viewing the pineal as an environmental sensor that enables the animal to adjust to seasonal changes. The advantage of being able to respond to these changes is obvious when one considers the reproductive aspects involved. The present study suggests that the pineal may be involved in an evaluation of the nutritional quality of the environment⁸.

Résumé. Le régime de contrôle (alimentation de laboratoire des lapins) de *Microtus montanus* a été augmenté de plantes vertes. La glande pinéale des animaux recevant le supplément eut un poids inférieur à celui de la glande des témoins ($p < 0,01$).

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Table II. Effect of daily dietary supplement of fresh green plants on pineal, reproductive tract and gonad weight in wild caught *Microtus montanus*

No. of animals	Sex	Treatment	\bar{x} pineal weight (mg)	\bar{x} reprod. tract. or gonad wt. (mg/g body wt.)
8	♀	Greens	0.080 \pm 0.054	50.0 \pm 32.8
8	♀	No greens	0.096 \pm 0.054	38.5 \pm 14.1
8	♂	Greens	0.046 \pm 0.047	0.95 \pm 0.54
8	♂	No greens	0.102 \pm 0.060	0.52 \pm 0.34

Animals were maintained under 8L 16D and were killed in the middle of the light period.

⁶ N. C. NEGUS and P. J. BERGER, unpublished results.

⁷ R. J. WURTMAN, J. AXELROD and J. E. FISCHER, *Science* 143, 1329 (1964). – W. B. QUAY, *Proc. Soc. exp. Biol. Med.* 121, 946 (1966).

⁸ This project was supported by the Atomic Energy Commission, contract No. AT-(40-1)-3946, the United States Public Health Service No. NIH-GM 00669-MBS and a Biomedical Sciences Support Grant from Tulane University.

Hepatic Δ^4 -Steroid Hydrogenase in the Pregnant Rat

Livers of pregnant rats have been shown to increase markedly in size, nucleic acid and protein content¹. A preferential increase in liver DNA has been observed during the second week of pregnancy¹ and there is evidence that this is due to proliferation of the cells of the reticulo-endothelial system². It has been reported that hepatic Δ^4 -steroid hydrogenase activity, the rate controlling step in corticosteroid inactivation in the rat³, is associated with the former cell type⁴. The present work was undertaken to determine the activity of hepatic Δ^4 -steroid hydrogenase during pregnancy in the rat since decreased levels of circulating corticosterone have been observed in pregnant rats from the 8th day of pregnancy to parturition⁵.

Methods. Virgin female Wistar rats, approximately 3 months old, were mated and together with virgin rats

of a similar body weight were fed daily 15 g of a diet containing 25% casein, 60% carbohydrate, 4% fat and adequate vitamin and mineral supplements⁶. Day 1 of pregnancy was assigned to the day following the observation of a plug or spermatozoa in the vagina. Groups of 3 or 4 pregnant rats were killed on the 10th, 12th, 14th, 16th, 18th and 20th day of pregnancy. The virgin rats were killed after they had been fed the diet for 10 or 18 days. The final body weight and liver weight of the non-pregnant rats were found to be independent of the length of time these animals were fed the diet. Δ^4 -steroid hydrogenase activity of liver slices was estimated by a method described previously⁷ and the in vitro synthesis of corticosteroids by adrenal slices was estimated as described by BAKKER and DE WIED⁸. Differences between groups were tested for significance by Student's *t*-test.