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 μg). 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.

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 ± 0.054	50.0 ± 32.8
8	♀	No greens	0.096 ± 0.054	38.5 ± 14.1
8	♂	Greens	0.046 ± 0.047	0.95 ± 0.54
8	♂	No greens	0.102 ± 0.060	0.52 ± 0.34

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

Dietary supplements of fresh green plant food have been shown to stimulate reproductive responses in *M. montanus*[5]. 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 animals[6]. Other workers have shown that HIOMT activity in the pineal is reduced during periods of light[7].

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 environment[8].

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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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 content[1]. A preferential increase in liver DNA has been observed during the second week of pregnancy[1] and there is evidence that this is due to proliferation of the cells of the reticulo-endothelial system[2]. It has been reported that hepatic Δ^4-steroid hydrogenase activity, the rate controlling step in corticosteroid inactivation in the rat[3], is associated with the former cell type[4]. 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 partuition[5].

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 supplements[6]. 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 previously[7] and the in vitro synthesis of corticosteroids by adrenal slices was estimated as described by BAKKER and DE WIED[8]. Differences between groups were tested for significance by Student's t-test.

Results and discussion. An increase in liver size relative to body weight was apparent by the 10th day of pregnancy (Figure a). After the 14th day, there was a further increase in liver weight to a level which was maintained until the 20th day ($P < 0.02$, for difference between mean liver weight on days 10–14 and mean liver weight on days 16–20). As seen in Figure b, a significant increase in Δ^4-steroid hydrogenase activity per g of liver occurred only on the 12th and 14th days of pregnancy ($P < 0.025$), coinciding with the period of liver growth when proliferation of reticulo-endothelial cells has been reported[2]. Total Δ^4-steroid hydrogenase activity was significantly elevated above the control level from the 12th day of pregnancy and may thus be a factor in maintaining lower levels of circulating corticosteroids at least during the later stages of pregnancy (Figure c).

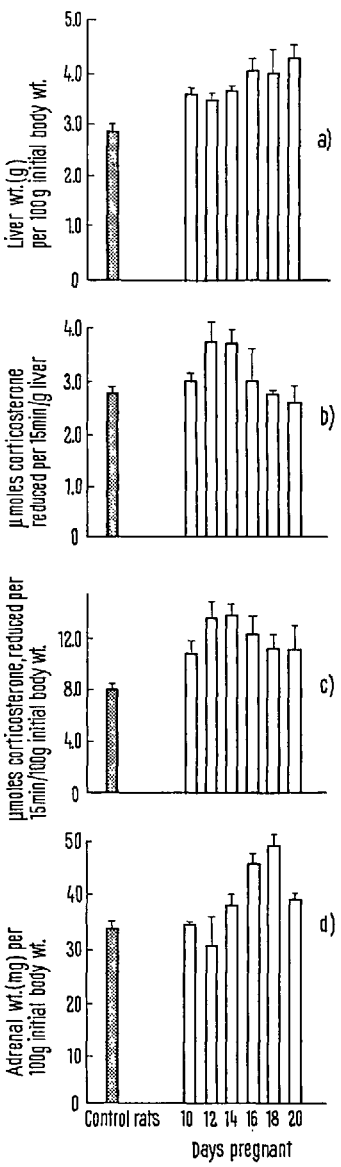
Administration of oestrogens to non-pregnant female rats has been shown to decrease the rate of corticosterone inactivation by decreasing the level of liver Δ^4-steroid hydrogenase activity[9]. In view of the occurrence of markedly increased levels of circulating oestrogens found during pregnancy in several species, the increased levels of hepatic Δ^4-steroid hydrogenase in the present work are of interest. However in the rat it would appear that there is no significant rise in plasma oestrogen level until the day preceding partuition[10].

There was no significant increase in adrenal weight until the 16th day of pregnancy, 4 days after the elevated level of hepatic steroid inactivation had become established (Figure d) and the level of synthetic activity of the adrenal gland in vitro was similar on the 12th, 14th and 18th days of pregnancy to that found in the non-pregnant animal and only on the 16th and 20th days were significant increases observed (Table). Thus there is little evidence to suggest any correlation between elevated hepatic Δ^4-steroid hydrogenase activity and increased corticotrophin secretion in the pregnant rat such as has been reported in other physiological and pathological conditions[3,7].

Corticosteroid synthesis by liver slices from pregnant rats

Days pregnant	µg Corticosteroid formed per 2 h/100 mg adrenal[a]	P[b] (I)	P[b] (II)
Control	2.82 ± 0.20	–	–
12	3.09 ± 0.54	n.s.	–
14	2.69 ± 0.29	n.s.	n.s.
16	4.43 ± 0.12	< 0.02	< 0.01
18	2.73 ± 0.17	n.s.	< 0.05
20	5.12 ± 0.16	< 0.005	< 0.02

Results are expressed as the mean of 3 experiments $\pm$ S.E. for the pregnant rats and the mean of 6 experiments $\pm$ S.E. for the control rats. [a] Calculated using corticosterone as reference standard. [b] I. Statistical significance of difference between pregnant and control groups. II. Statistical significance of difference between a pregnant group and the preceding pregnant group.



Variation of (a) liver weight, (b) Δ^4-steroid hydrogenase activity per g liver, (c) total hepatic Δ^4-steroid hydrogenase activity and (d) adrenal weight from the 10th to 20th day of pregnancy. Values are the mean $\pm$ S.E. of 7 rats for the control group and the mean $\pm$ S.E. of 3 or 4 rats for the pregnant groups.

Résumé. Chez la rate gravide, la réduction du corticosterone, exprimé par gramme de foie fut augmentée les douzièmes et quatorzième jours de gestation. L'activité du foie entier augmenta à partir du douzième jour.

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