processes, i.e. further enhancing progesterone and oestradiol levels while they are high, though not by preventing their decline nor advancing their rise. Consequently, the pineal may be able to modify the levels of gonadal hormones but not affect the sequence of the physiological events. In a previous study[3] in which we investigated changes in pituitary hormones during pregnancy in pinealectomized rats, an enhanced increase in serum LH and decreased serum prolactin rise were observed during the 2 days before parturition. Thus, in the absence of the pineal, the increase in the gonadal hormone progesterone precedes the changes in pituitary hormone levels, which would rule out a direct effect of the pituitary on progesterone secretion. Moreover the enhanced levels of LH in serum do not affect the timing of the sharp decline in progesterone that occurs in the same animals at the end of pregnancy. This is in accord with the proposal made by Gibori and Richards[4], that in the 2nd half of pregancny the pituitary has an inhibitory effect on luteal cell function and that this may be being regulated by a placental-pituitary feedback. They suggest that the placenta may be regulating corpus luteal cell LH receptor content and progesterone production in the 2nd half of pregnancy by 2 of its hormones, 1 luteotrophic prolactin-like, the other LH-like chorionic gonadotrophin. The increased progesterone levels induced by pinealectomy may therefore result from enhanced production of placental hormones, as has been indicated by the prolactin-like luteotrophin which is at its peak on day 18 or pregnancy[5].

Furthermore, it was demonstrated that during the prenatal period adrenal steroidogenesis contributes significant amounts of progesterone to the total maternal pool[6]. On day 22 of pregnancy, adrenal secretion of progesterone reaches a level severalfold that produced by the ovary or at any time during the oestrous cycle. Since the pineal gland is functioning as an inhibitory modulator of the adrenal cortex in rats with normal oestrous cycle[7], it is possible that the enhanced adrenal function brought about by pinealectomy is largely responsible for the increased progesterone levels in pregnant pinealectomized rats.

The sharp decline in progesterone that takes place ante partum in pinealectomized rats coincides with a surge of LH[3]. The possibility exists that the higher levels of LH in serum of pinealectomized animals contribute to the lowering of the progesterone peaks, bringing them down on day 21 to levels almost as low as those of the controls. This postulation may be substantiated by the finding that the ante partum fall in serum progesterone requires the presence of the pituitary[8].

Regarding the changes in oestradiol brought about by pinealectomy, it seems likely that the pineal hormones normally exert an inhibitory effect on oestrogen production via the placental-pituitary complex mentioned previously. The pinealectomy-potentiated increase in serum oestradiol levels during the period preceding parturition coincides with the enhanced rise in serum LH[3] and could be a result of a positive feedback of that hormone.

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1 L.C. Ellis, Am. Zool. *16*, 67 (1976).
2 K.P. Minneman and R.J. Wurtman, A. Rev. Pharmac. Toxic. *16*, 33 (1976).
3 I. Nir, N. Hirschmann, G. Goldhaber and J. Shani, Neuroendocrinolony *28*, 44 (1979).
4 G. Gibori and J.S. Richards, Endocrinology *102*, 767 (1978).
5 P.A. Kelly, R.P. Shiu, M.C. Robertson and H.G. Friesen, Endocrinology *96*, 1187 (1975).
6 T.F. Ogle and J.I. Kitay, J. Endocr. *74*, 89 (1977).
7 I. Nir, in: The Pineal Gland, p.225. Ed. I. Nir, R.J. Reiter and R.J. Wurtman. Springer-Verlag, Wien 1978.
8 G. Pepe and I. Rothchild, Endocrinology *91*, 1380 (1972).

The effects of vitamin E-deficiency on serum prolactin and serum luteinising hormone levels in the pregnant rat[1]

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Summary. No significant differences were observed between the serum prolactin or serum LH levels of vitamin E-deficient or vitamin E-replete rats during the first 12 days of gestation. It is suggested that pituitary dysfunction is not the cause of the characteristic foetal resorption observed in vitamin E-deficient rats.

Vitamin E-deficiency in the female rat is characterized by foetal resorption[3] which can be prevented by administration of adequate doses of the vitamin as late as the 11th day of the pregnancy[4]. Since hypophysectomy in the rat up to day 11 of gestation also results in termination of the pregnancy[5], it is not surprising that several investigators have sought to explain the characteristic foetal resorption in vitamin E-deficient rats in terms of pituitary dysfunction. However, the results of such investigations have provided conflicting results. Thus Nelson[6] observed no histological differences between the pituitaries of vitamin E-deficient or vitamin E-replete rats at various stages of gestation. In contrast, Barrie[7] reported a degranulation of the basophils of the pituitary in vitamin E-deficient rats suggesting a decreased gonadotrophin concentration. Similarly, estimations of the gonadotrophin content of the pituitary of pregnant vitamin E-deficient rats by biological assay have indicated that the concentration is decreased[8], increased[9,10], or is unchanged[11]. During the first half of gestation in the rat, the corpus luteum is maintained by the actions of the pituitary hormones prolactin and luteinising hormone (LH)[12-16]. The present study was undertaken to establish whether or not foetal resorption in the vitamin E-deficient rat is associated with changes in the circulating levels of prolactin and LH.

Materials and methods. The animals used in this study were female wistar rats bred in this laboratory. An experimental group of rats were weaned at 21 days of age on a vitamin E-deficient diet composed of purified casein 25%, dried yeast 10%, lard 5%, codliver oil 5%, sucrose 49%, vitamin premix (no vitamin E) 1%, and a mineral premix 5% (Cooper Nutrition Ltd.). A control group of animals were weaned on an identical diet with the exception that vitamin E was added at a concentration of 250 IU/kg. All animals were housed individually in a controlled environment (22 °C; 14/10 light/dark schedule), and allowed access to food and

water ad libitum. Preliminary studies showed that female rats fed the vitamin E-deficient diet for 120 days after weaning, while showing a normal oestrus cycle exhibited the classical symptoms of foetal resorption, whereas those animals fed the control diet underwent a normal pregnancy.

120 days after being placed on either the vitamin E-deficient or control diets the female rats were mated with proven males of the same strain, and detection of sperm in vaginal smears was designated day 1 of the pregnancy. Blood samples ($\triangleq$1 ml) were collected by heart puncture under light anaesthesia on days 1, 6 and 12 of the pregnancy between 10.00 and 11.00 h.

In an attempt to reduce variations in the circulating levels of LH and prolactin due to differences in the antepartum interval, blood samples from those animals which did not exhibit a placental sign of implantation on day 13 of the pregnancy were discarded. In addition, blood samples from any females fed the vitamin E-deficient diet which delivered viable young were similarly discarded. Foetal resorption in the remainder of the vitamin E-deficient group was confirmed by examination of the uteri on day 22 of the pregnancy.

Blood samples were allowed to clot at 4 °C, centrifuged at 3000 rev/min and the serum separated and frozen at -20 °C until assayed. Prolactin and LH concentrations were assayed at 2 dose levels by a double antibody radioimmunoassay as recommended in the directions supplied with RIA-NIAMD kits. Serum prolactin levels are expressed in terms of NIAMD-rat prolactin-RP-1 (biological potency $\triangleq$11 IU/mg) and LH in terms of NIAMD-rat LH-RP-1 (biological potency = 0.03 × NIH-LH-SH, OAAD assay). The significance of differences between groups was calculated by Student's t-test.

Results and discussion. Details of the circulating levels of LH during the first 12 days of pregnancy in the vitamin E-deficient and vitamin E-replete rats are summarized in figure 1. In both groups of animals serum LH levels decreased significantly between day 1 and day 6 ($p < 0.01$), and then remained relatively constant during the remaining 6 days of the experiment. Although the serum LH levels of the vitamin E-deficient rats were consistently lower than those of the replete animals on the days of sampling, there was no significant difference ($p > 0.05$) between the unweighted means of the deficient (1.59 ± 0.19 ng/ml) and replete group (2.02 ± 0.18 ng/ml) over the experimental period. Similarly, there was no significant difference between the serum LH levels of vitamin E-deficient and vitamin E-replete rats on days 1 and 6 of the pregnancy ($p > 0.05$) However, on the 12th day of gestation the serum LH levels of the vitamin E-deficient group were significantly lower than those of the replete group ($p < 0.05$), but it is precisely at this time that the pituitary becomes dispensable to the maintenance of pregnancy in the rat[4]. These results support the suggestion that the changes in pituitary function in the pregnant vitamin E-deficient rat are secondary phenomena[3,10], and are not responsible for foetal resorption per se.

Figure 2 summarises the serum prolactin levels of vitamin E-replete and vitamin E-deficient rats during the first 12 days of pregnancy. In both groups of animals serum prolactin levels were highest on day 1 of the pregnancy and then decreased progressively over the next 11 days. Like the serum LH levels, the serum prolactin concentration of the vitamin E-deficient group was consistently lower than those of the replete group at the times of sampling. However, no significant difference ($p > 0.05$) was observed between the unweighted means of the vitamin E-deficient (16.19 ± 2.65 ng/ml) or vitamin E-replete (20.77 ± 2.75 ng/ml) rats over the 12 day experimental period. Similarly a comparison of the serum prolactin levels on days 1, 6 and 12 of gestation revealed no significant differences ($p > 0.05$) between the 2 groups of rats.

The results indicate that the circulating levels of prolactin and LH in the vitamin E-deficient pregnant rat are slightly, but not significantly, lower than those found in rats receiving an adequate dietary source of the vitamin during the first 12 days of pregnancy. Consequently, it would appear that there is no impairment in the production or secretion of either prolactin or LH in the vitamin E-deficient rat, suggesting that pituitary dysfunction is not the cause of foetal resorption in these animals.

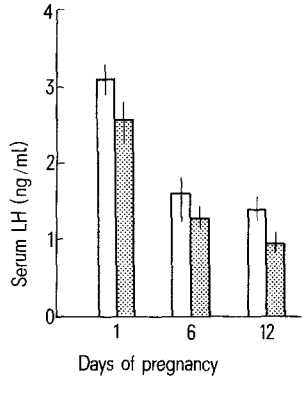

Fig. 1. Serum LH levels of vitamin E-replete (□) and vitamin E-deficient (■) rats during the first 12 days of pregnancy.

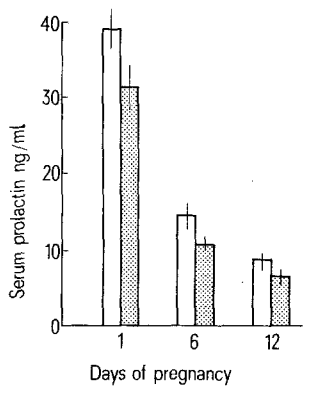

Fig. 2. Serum prolactin levels of vitamin E-replete (□) and vitamin E-deficient (■) rats during the first 12 days of pregnancy.

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2 Pharmacia (Great Britain Ltd) Hounslow, Middlesex.
3 H.M. Evans and G.O. Burr, Mem. Univ. Calif. *8*, 1 (1927).
4 K.E. Mason, Vitam. Horm. *2*, 107 (1946).
5 R.I. Pencharz and J.A. Long, Am. J. Anat. *53*, 117 (1933).
6 W.O. Nelson, Anat. Rec. *56*, 241 (1933).
7 M.M.O. Barrie, Lancet *11*, 251 (1937).
8 I.W. Rowlands and E. Singer, J. Physiol. *86*, 323 (1936).
9 S.Y. P'An, H.B. van Dyke, H. Kaunitz and C.A. Slanetz, Proc. Soc. exp. Biol. Med. *72*, 523 (1949).
10 W.E. Griesbach, M.E. Bell and M. Livingston, Endocrinology *60*, 729 (1957).
11 C. Biddulph and R.K. Meyer, Am. J. Physiol. *132*, 259 (1941).
12 G.S. Greenwald and I. Rothchild, Animal Sci. *27*, suppl. 1, 139 (1967).
13 J.J. Alloiteau and J. Bouhours, C.r. *261*, 4230 (1965).
14 K. Loewit, Acta endocr. *65*, suppl. 149 (1970).
15 H.G.M. Raj and N.R. Moudgal, Endocrinology *86*, 874 (1970).
16 D.T. Armstrong, J. Reprod. Fert. suppl. 1 (1966).