

Hormonal Control of "Delayed Development" in *Macrotus waterhousii*¹

I. Changes in Plasma Thyroxine during Pregnancy and Lactation

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Females in various stages of reproduction were collected from southern Arizona. Pregnant animals were placed in a controlled environment and given an ample diet of mealworms and crickets. This new environment had no effect on the gestation period, and pregnancy proceeded normally for 7 months. Plasma thyroxine concentration and histological sections of the thyroid were studied from animals in various stages of pregnancy and during lactation. Plasma thyroxine (T_4) was highest during the last trimester of pregnancy (5.4 $\mu\text{g}/100\text{ ml}$) and lactation (5.2 μg) and lowest during the first and second trimester (2.0 and 1.9 μg) of the 9-month gestation period. No correlation was found between thyroid follicle dimensions and the fluctuation in plasma thyroxine.

Of the several described patterns of mammalian reproduction, only the funnel-eared bat, *Natalus stramineus*, and the leaf-nosed bat, *Macrotus waterhousii* (= *M. californicus*), are known to exhibit what has been termed "delayed development." In contradiction to delayed implantation and delayed fertilization (see Daniel, 1970), delayed development is characterized by immediate fertilization and implantation followed by a greatly reduced rate of embryological development. For purposes of comparison, in most small mammals of similar weight (10-30 g) the gestation period is less than 30 days (Asdell, 1964). In most bats of comparable size, the gestation period is somewhat longer (approximately 3 months). However, in *Natalus stramineus* the gestation period is 8-10 months (Mitchell, 1965), and in *Macrotus waterhousii* the gestation period is approximately 9 months (Bradshaw, 1962). A process of delayed develop-

ment has also been suggested for the vampire bat, *Desmodus rotundus*, which has a gestation period of 5 months and maybe longer (Wimsatt and Trapido, 1952) and for *Artibeus jamaicensis* (Fleming, 1971). The hormonal changes to accommodate such a reproductive pattern are unknown. In our laboratory, *Macrotus waterhousii* was chosen as the species for investigation of hormonal regulation of delayed development because it is more readily available, more durable, and twice the size of *Natalus*.

Our first investigations into possible regulatory mechanisms of delayed development were to follow up the work of Bradshaw (1962). In this study it was shown that in *Macrotus waterhousii* copulation and implantation occurred during September to November. In the first 6 months of pregnancy there was little development of the embryo. From late March until June, however, there was a more rapid rate of development approaching the normal rate for other bat embryos of comparable size. Bradshaw proposed two factors, low winter

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temperatures and food shortage, which he believed caused this lengthy diapause in the development of the embryo.

The purpose of this study was to test Bradshaw's hypothesis and also to monitor thyroid activity because of the importance of thyroid hormones in embryonic growth and pregnancy (Myant, 1964; Marks *et al.*, 1966).

METHODS

Male and female *M. waterhousii* were collected in late October and early November from various abandoned mine sites in southern Arizona. The animals were transported to environmental chambers at Texas Tech which were equipped with temperature (24°C) and humidity (approximately 80%) controls. The animals were maintained on a photoperiod of 14 hr light-10 hr dark (lights on at 6:00 AM until 8:00 PM). Mealworms, crickets, and water were placed in each cage. After a period of about 3 days during which the animals had to be hand-fed, the bats were allowed to feed ad libitum.

After approximately 3 months, the bats were transferred to a specially modified greenhouse (9'8" × 27'7"). The greenhouse was equipped with temperature control (maintained at 24°C) and allowed the bats considerable freedom of flight. Mealworms and water were placed on a centrally located table, and the bats readily fed ad libitum. Appropriate vegetation was added so that the "bat room" had a semitropical environment.

At regular intervals during the gestation period, a number of pregnant bats from natural populations and the laboratory colony were exsanguinated by decapitation. All animals were routinely sacrificed during the afternoon between 1:00 and 4:00 PM. The blood of three individuals was pooled in order to obtain a sufficient amount of serum (1 ml) for the thyroxine assay. Thyroxine assays were performed by the method of Pileggi *et al.* (1961) via a specially designed kit obtained from Oxford Laboratories, San Mateo, California.

Immediately after sacrifice, the reproductive tracts and thyroids were removed. These organs were fixed in AFA (acetic acid, formalin, and ethyl alcohol), embedded in paraffin, sectioned at 10 μ , and stained with hematoxylin and eosin. The thyroid follicles were measured noting follicle diameter, colloid diameter, and follicle cell height. These data were recorded and used as an additional means of evaluating thyroid activity. The sections of the reproductive tracts were stored for future studies to establish accurate embryo growth-time relationships.

RESULTS

In order to test Bradshaw's original hypothesis that reduced food supply and winter temperature were the cause of the embryonic diapause, 12 pregnant animals were placed in environmental chambers with temperature and humidity controls. In addition, the animals were fed a more than sufficient diet of mealworms and crickets. All females responded well to the new environment and gained weight on the average from an initial 10 g to 13 g. Three months later, the animals were transferred to the modified greenhouse, and an additional 3 g average weight increase was recorded. Examination of two animals at this time, however, revealed that the uterine size did not indicate an advanced stage of development and corresponded to the growth pattern witnessed for several dozen animals in the same stage of pregnancy taken from natural populations in southern Arizona.

The lab colony was maintained until May 11, at which time a tornado demolished the greenhouse. All the bats either escaped or were killed in the disaster. Autopsy of three dead females revealed that after 6 months of ideal temperature and abundant food supply, the embryonic growth was comparable to that of bats collected in the field. All laboratory females appeared to be in the latter stages of pregnancy (swollen abdomen) before the tornado.

The results of the plasma thyroxine study during each stage of reproduction are presented in Fig. 1. Each point depicts the average T_4 concentration of 15 animals. The values are equivalent to $\mu\text{g } T_4/100 \text{ ml}$ of plasma. The data were analyzed using a complete random design analysis of variance (Steel and Torrey, 1960). Since the F test was significant ($F_{5,84} = 189.86$, $p = < 0.001$), the statistical analysis was expanded to include Duncan's Multiple Range Test for mean separation. This analysis showed that there was no significant difference between means A and B (immature and nonpregnant adult); however, all other combinations of means were different ($p = < 0.01$).

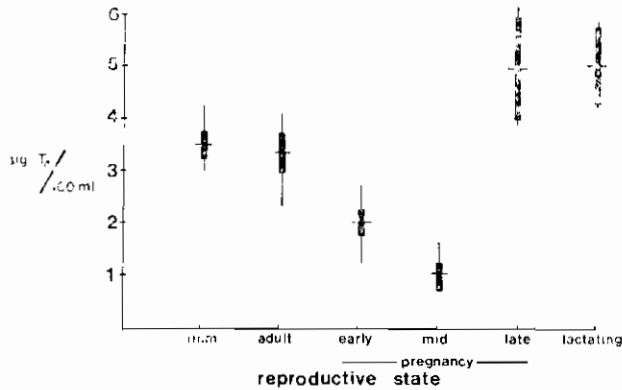


FIG. 1. Plasma thyroxine concentration of leaf-nosed bats in various stages of reproduction. Values presented represent the average of 15 animals (horizontal line), the range of each group (vertical line), and \pm two standard errors of the mean (vertical bar).

The results of thyroid histological examination are summarized in Table 1. Thyroids from 3 animals in each group were sectioned. Data are based on 10 randomly selected follicles from each thyroid. Analysis of variance for each character (i.e., weight, follicle diameter, etc.) was performed; however, in every case the F value was not significant, and no further analysis was performed.

The thyroid sections prepared from animals in the last trimester of pregnancy contained 3 or 4 exceptionally large follicles (110μ). No sections from any other reproductive stage reached any such magnitude (the closest being 75μ from a lactating individual).

DISCUSSION

The fact that a constant temperature had little effect on accelerating embryonic growth is not surprising when one considers the natural habitat of *Macrotus water-*

housii. These animals characteristically inhabit abandoned mine shafts in southern Arizona which remain quite warm (approximately 27°C) and constant throughout the year. Even though the females venture forth at night during the mild winters to feed, they have a stable, warm environment to return to each morning.

The drastic fall in plasma thyroxine represents what may be a unique pattern in nonhibernating animals. Several reports have pointed out conflicting data concerning T_4 levels in the blood during pregnancy. In rats, for example, some reports indicate no change in T_4 , while others report a slight increase in circulating levels of the hormone (Zarrow, 1961). Recent reports using resin column assay techniques similar to those mentioned in this study suggest that, in human pregnancy, the thyroxine concentration may be reduced but the total PBI doubles to as much as $12 \mu\text{g}/100 \text{ ml}$ (Ingbar and Woeber, 1968). It should also be men-

TABLE 1
A COMPARISON OF THYROID SIZE FROM LEAF-NOSED BATS IN VARIOUS STAGES OF REPRODUCTION

| Reproductive state | Thyroid weight ^a (mg) | Follicle di- ameter (μ) | Colloid di- ameter (μ) | Epithelial cell height (μ) |
|--------------------|-------------------------------------|----------------------------------|---------------------------------|-------------------------------------|
| Immature | 3.2 | 49 | 39 | 5.0 |
| Adult nonpregnant | 4.0 | 49 | 41 | 5.1 |
| Mid-pregnant | 4.2 | 54 | 43 | 5.7 |
| Late pregnant | 4.1 | 51 | 40 | 5.5 |
| Lactating | 4.7 | 54 | 43 | 5.7 |

^a Average weight; N for each reproductive state = 15.

tioned, however, that this assay measures approximately 90% of the total thyroid hormones in the plasma.

Since there was such a drastic fluctuation in plasma T_4 , we felt that there might also be a similar change in the histology of the thyroid gland. However, no differences were found and the absence of change in thyroid dimensions during pregnancy is in keeping with the reports of numerous other investigators (see review by Myant, 1964).

At this point, perhaps it would be germane to mention how the reproductive state of animals was determined. Basically, we relied on Bradshaw's work, on histological surveys of reproductive tracts from animals with known capture dates and on the degree of abdominal swelling. In most cases these observations were confirmed during latter autopsies. However, it should be pointed out that the embryology of these animals is not well known. Consequently, there are no accurate growth curves or embryonic markers one can use to properly classify the reproductive state. Our system is certainly arbitrary at best.

Many reports are available concerning the effects of antithyroid drugs and thyroidectomy on pregnancy. In the rat, litter size, as well as the quality of each member of the litter is seriously affected by hypothyroid mothers (Zarrow, 1961). It has also been shown that treatment with thiouracil can extensively extend the gestation of the rat and mouse (Johnson and Meites, 1950).

The mechanisms of thyroid hormone action on reproductive processes are far from clear. The answers at present appear to be bound in a maze of sophisticated and contradictory biochemical observations (see Ingbar and Woebber, 1968, for reviews). However, it does appear that a euthyroid condition in the mother is essential to normal pregnancy and that a hypothyroid condition due to either artificial or natural dysfunction of the gland can prolong the normal gestation period.

Again, it must be emphasized that *Macrotus waterhousii* is a nonmigratory and nonhibernating species. Consequently, the reports concerning periodic reduction in thyroid activity seen in hibernating animals

have no major importance to this particular study except to point out that the changes are somewhat similar (see review by Kayser, 1965).

It thus appears that in *Macrotus waterhousii* we may have an example of a system in which the maternal thyroid is naturally shut down during the early stages of pregnancy. This temporary hypothyroidism is probably the functional end point through which a complicated and highly integrated endocrine control system is manifested. A brief insight into the complexity of this system, for example, is that we injected 5 μ g of T_4 per day for 20 days into pregnant females and failed to increase the rate of embryonic development. Further studies along this line are in progress. In addition, we plan to attempt to induce further prolongation of gestation by antithyroid drug treatment during the normally accelerated growth phase of the last trimester of pregnancy.

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