relation between Mycoplasma antibody and the normal enhancing factor in serum.

1. Adler, H. E., DaMassa, A. J., Avian Dis., 1965, v9, 205.
2. ———, Proc. Soc. Exp. Biol. Med., 1964, v116, 608.
3. Adler, H. E., DaMassa, A. J., Sadler, W. W., Avian Dis., 1964, v8, 576.
4. Campbell, D. H., Garvey, J. S., Cremer, N. E., Sussdorf, D. H., W. A. Benjamin, Inc., 1964, 263.
5. Makinodan, T., Gengozian, N., Canning, R. E., J. Immunol., 1960, v85, 439.
6. Orlans, E., Rose, M. E., Clapp, K. H., Immunology, 1962, v5, 649.
7. Orlans, E., Rose, M. E., ibid., 1965, v8, 193.

Received November 4, 1966. P.S.E.B.M., 1967, v124.

Thyroid Effect on Birthweight in C 57 BL Mice and *Peromyscus*.* (31925)

RUTH E. WALTON AND WALLACE D. DAWSON (Introduced by B. T. Cole)
Department of Biology, University of South Carolina, Columbia

Conflicting evidence exists concerning the influence of thyroid hormones on birthweight in mammals. Thyroid hormone administered to pregnant females is reported to increase birthweight in rabbits(1), but decrease it in guinea pigs(2) and possibly rats(3). There are reports that goitrogens administered during pregnancy decreased fetal weight in rats(3,4) and birthweight in the guinea pigs (5). Maternal thyroidectomy reportedly reduced birthweight in C 57 mice(6), but other findings suggest that neither thiourea treatment nor thyroidectomy of pregnant rats materially effected neonatal weight(7,8).

To date there have been no attempts to critically evaluate the influence of physiological levels of maternal thyroid hormone on birthweight, particularly with reference to a known thyroid secretion rate (TSR) estimate. This prompted us to examine the effect of thyroid hormone upon weight of newborn in 3 forms of rodents where TSR data were available: C 57 BL laboratory mice *(Mus musculus);* deermice *(Peromyscus maniculatus bairdii);* and oldfield mice *(P. polionotus).* If the thyroid contributes significantly to birthweight in these rodents, experimental alterations of hormone levels within a subtoxic range should produce a measurable effect.

Materials and methods. The experimental procedures with C 57 BL mice were somewhat different from those with *Peromyscus.* This was necessitated by inherent differences in the stocks, particularly with regard to genetic variability. Further, it was thought that different approaches to the same problem might render the findings more conclusive.

C 57 BL mice of the 6 J line were employed because of the vigor, low tumor incidence and genetic homogeneity of the strain. Average adult TSR's estimated for this strain by the I^{131} depletion method(9) and by goiter prevention technique(10) vary from 3.33 to 4.80 μg l-thyroxine/100 g bw/day.

Nineteen mated C 57 BL pairs were established using 90-120-day-old animals. Pairs were divided into 4 groups, maintained under controlled conditions of light and temperature, fed and watered *ad libitum.* Pregnant mice from Group 1 received daily injections of l-thyroxine solution s.q. at the estimated normal female TSR (3.33 μg/100 g bw), and those in Group 2 received twice the estimated TSR. In both groups endogenous thyroid function was blocked utilizing .01% propylthiouracil (PTU) solution as drinking water. Pregnant mice in Group 3 were given PTU only, and those in Group 4 received a sham injection of distilled water daily. Only the second and third litters from each mated pair were used to obtain birthweight data.

Two varieties of *Peromyscus* were laboratory bred from mice 10 or more generations

*Supported by grant HD 01055 from Child Health and Human Development Council, Nat. Inst. Health.

TABLE I. Mean Birthweights of C 57 BL Mice from Mothers Treated with l-Thyroxine and/or Propylthiouracil to Modify Thyroid Hormone Levels.

Group	Birthweights (g) All litters	Litters 5, 6, 7	Litters 8, 9, 10
(1) 1 × TSR	1.30 ± .02 (65)*	1.33 ± .03 (28)	1.27 ± .02 (33)
(2) 2 × TSR	1.35 ± .02 (70)	1.40 ± .02 (38)	1.26 ± .05 (28)
(3) PTU	1.35 ± .02 (24)	1.37 ± .03 (16)	1.33 ± .03 (8)
(4) Control	1.32 ± .01 (66)	1.34 ± .02 (33)	1.30 ± .02 (33)

* Mean ± SEM (No. newborn).

removed from wild founder stocks. The *P. maniculatus* stock originated from animals collected in southern Michigan, while the *P. polionotus* stock was derived from mice from central Florida. Neither stock was highly inbred. Mean seasonal TSR's for *P. maniculatus* vary from 0.81 to 1.93 μg l-thyroxine/100 g bw/day(11) with an average mean value of 1.5 μg.

Fourteen *P. maniculatus* and nine *P. polionotus* stock pairs of healthy adult animals were utilized. These pairings had been previously established and were reproducing at regular intervals from post-partum conceptions. Experimental litters of both *Peromyscus* species were obtained from gravid mice injected with sodium l-thyroxine s.q. at five times the average mean normal TSR for *P. maniculatus* (5 × 1.5 μg/100 g bw/day). Control litters were obtained from the same mated pairs prior to treatment, *i.e.*, earlier litters were used as controls, subsequent litters as experimentals. Both control and experimental groups consisted of one to three litters obtained from each pair. This experiment was conducted during the active breeding season, April through mid-August.

Pregnancies in C 57 BL and *Peromyscus* were detected 7-12 days prior to parturition, and treatment was initiated at this time. Mated pairs were inspected between 4 and 5 PM each day for new litters and pregnancies, at which time treatment was administered and newborn were weighed individually to the nearest .01 g.

Results. Results of the experiments with C 57 BL mice are summarized in Table I. As anticipated, mean birthweights of Group 1 (1 × TSR) and Group 4 (Control) did not differ significantly. Conversely, these two groups might be expected to differ from Group 2 (2 × TSR) and Group 3 (PTU) if altered hormone levels were exerting an effect on newborn body weight. However, no significant differences could be demonstrated in mean birthweights between either Group 2 or 3 compared with Group 1 or 4 ($P>.05$).

Since birthweights are known to vary with litter size, all C 57 BL litters were assigned by size into categories of 5, 6 and 7 or 8, 9, and 10 offspring respectively. As before, with either of these litter size categories no significant differences were found between mean weights of any of the treated groups and the control ($P>.05$). Nevertheless, there was a highly significant difference, independent of treatment, between the mean birthweights of combined litters of 5, 6 and 7 compared with those of 8, 9 and 10 ($P<.01$). There was no strong evidence that any of the treatments materially modified litter size, although the data were not conclusive in the case of PTU treatment.

Table II shows the results of the investigation for *Peromyscus*. When animals from mothers which received 5 × the normal mean TSR were contrasted with siblings from untreated females again no significant differences in birthweight were detected ($P>.05$). The results from *Peromyscus* thus reinforced the findings from the C 57 BL experiment.

TABLE II. Mean Weights of Newborn *Peromyscus* from Mothers Treated with l-Thyroxine Contrasted with Previous Newborn from the Same Mothers Without Treatment.

Species	No. sibships	Birthweight (g) Untreated	5 × TSR
P. maniculatus	14	1.69 ± .01* (141)	1.66 ± .01 (142)
P. polionotus	9	1.58 ± .02 (69)	1.61 ± .02 (64)

* Mean ± SEM (No. newborn).

Discussion. The experiments reported here support the view that moderate levels of maternal circulating thyroid hormones have no effect on birthweight in these rodents. Neither the virtual absence of these hormones in the PTU treated mice nor excess dosages up to 5 times the normal secretion rate produced a detectable effect on neonatal weight of offspring. There is ample evidence that thyroxine traverses the placenta in rodents, particularly late in gestation, as does PTU (12,13), which would negate assumptions that maternal thyroxine does not reach the fetus or that there is a compensatory action by the fetal thyroid. The conclusion that the fetus in these species fails to elicit a growth response to thyroid hormone is in marked contrast to pronounced growth inhibition in hypothyroid postnatal animals. This is consistent with the studies of Shapiro(14) with the rat, which demonstrated that early postnatal modifications of thyroid function had little effect on growth, but that thyroid function became increasingly important as the animal approached weaning age. The data of Carteret and Delost(15), which indicated thyroxine administered to gravid mice had no effect on the weight of 10-day-old offspring, lends further substantiation, as do the previously cited reports that thyroidectomy and thiourea treatment of females frequently fails to materially alter the weight of their progeny. On the other hand, the role of thyroid hormones in other aspects of fetal development, *e.g.*, brain maturation, are not necessarily obviated by these findings. In the present investigation we noted that dermal pigmentation appeared to be accelerated in offspring from thyroxine-treated *P. polionotus*.

Workers who found thyroid-treated mothers produced smaller progeny, in most instances, were using dosage levels many times the normal secretion rate. At these high dosages the reduced birthweight may have been due to thyrotoxic effects of excessive hormone. Since it would be difficult to conceive of untreated animals with the capacity to produce thyroid hormones at such high levels, the relevance of such findings to normal development is not apparent. The report by Theresa(1) that orally administered thyroid enhanced the size of offspring in rabbits is difficult to reconcile with our conclusions unless there is a species difference in fetal growth response.

Summary. Thyroxine and/or propylthiouracil were administered to pregnant C 57 BL mice, *Peromyscus maniculatus* and *P. polionotus* to alter the thyroid hormone titer. No significant differences in birthweight were observed between offspring from mothers receiving either thyroxine in excess of the normal secretion rate or receiving thiouracil treatment contrasted with controls. Sub-toxic levels of maternal or fetal thyroid hormone appear to have no marked effect on neonatal weight in these rodents.

The technical assistance of James Kelly is gratefully acknowledged.

1. Theresa, S., Bull. Biol. & Med. Exp. USSR, 1939, v7, 544.

2. Siedentopf, H., Monatsschr. Geburtsh. u. Gynakol., 1930, v85, 325.

3. Noumura, T., Jap. J. Zool., 1959, v12, 301.

4. Jost, A., Moreau, G., Fournier, C., Arch. Anat. Microscop. Morphol. Exp., 1960, v49, 431.

5. D'Angelo, S. A., Proc. Soc. Exp. Biol. & Med., 1966, v121, 555.

6. Davenport, C. B., Swingle, W. W., J. Exp. Zool., 1927, v48, 395.

7. Goldsmith, E. D., Gordon, A. S., Charipper, H. A., Am. J. Obst. & Gyn., 1945, v49, 197.

8. Langman, J., Van Faassen, F., Acta Physiol. Pharmacol. Neerl., 1955, v4, 109.

9. Amin, A., Chai, C. K., Reineke, E. P., Am. J. Physiol., 1957, v191, 34.

10. Chai, C. K., J. Hered., 1958, v49, 143.

11. Eleftheriou, B. E., Zarrow, M. X., Proc. Soc. Exp. Biol. & Med., 1962, v110, 128.

12. Mitskevitch, M. S., Gen. and Comp. Endocrin., 1962, Suppl. 1, 300.

13. Contopoulos, A. N., Ryan, M., Contopoulos, C. R., Amoroso, E. C., Proc. Zool. Soc. London, 1964, v142, 699.

14. Shapiro, S., Endocrinology, 1966, v78, 527.

15. Carteret, P., Delost, P., C. R. Soc. Biol., 1961, v155, 2334.

Received December 12, 1966. P.S.E.B.M., 1967, v124.