Hobbins: That is interesting. One wonders what turns it on. The anencephalic discussion also is interesting in this regard.

Greer: It wasn't clear as to what the anencephalic reference meant.

Sack: You mentioned that there seemed to be evidence of hypothalamic maturation before birth, that you could see a reaction to LID and to PTU in the fetus. Then Dr. Dussault showed that the maturation normally seems to occur after birth. I mentioned the human anencephalic infant without a brain or a hypothalamus in which the pituitary is responsive after birth; there is measurable TSH in the serum. Perhaps an independent pituitary is reacting to something other than TRH.

Greer: But there is some evidence that anencephalics do not have much TSH coming out. Dr. Fisher and our group collaborated (Allen et al., *J. Clin. Endocrinol. Metab.*, 38:94, 1974) in studying one patient whose TSH was very low and did not surge after birth. The infant responded well to TRH administration with a marked rise in plasma TSH.

Sack: Again it suggests that the infant did not have TRH *in utero*. We studied an anencephalic infant with 6 μU/ml of TSH in the serum at birth. We did not measure TSH in the pituitary, but it seems that the pituitary of at least some anencephalic infants can manufacture TSH and release it into the serum.

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Endocrine Syndromes Produced by Neonatal Hyperthyroidism, Hypothyroidism, or Altered Nutrition and Effects Seen in Untreated Progeny

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I. LATE EFFECTS OF NEONATAL HYPERTHYROIDISM ON CERTAIN ENDOCRINE REGULATORY SYSTEMS IN THE ADULT RAT

The administration of androgens to the neonatal female rat produces persistent alterations in hypothalamic centers regulating pituitary gonadotropin secretion (Barraclough, 1961; Harris, 1964). With this syndrome and the experiments of Eayrs and Holmes (1964) as a model, we have studied the late and persistent effects of administering large doses of sodium L-thyroxine (T4) to the neonatal rat during the first 5 days of life. This treatment resulted in impairment of thyroid and pituitary growth, often disproportionately greater than the impairment in body growth (Bakke and Lawrence, 1966), and plasma concentrations of protein-bound iodine and free T4 (FT4) were reduced (Gellert, Bakke, and Lawrence, 1971). Such animals also exhibited delayed puberty as indicated by late vaginal opening and first estrus, prolonged cycles (Gellert et al., 1971), reduced ovarian weight (Bakke, Lawrence, and Robinson, 1972), and impaired fertility (Bakke and Lawrence, 1968). The pituitary thyrotropin (thyroid stimulating hormone, TSH) content was diminished (Bakke and Lawrence, 1966), and the stalk median eminence (SME) TSH content was elevated (Bakke and Lawrence, 1967). When challenged with propylthiouracil (PTU) as adults, there was significantly impaired goiter growth, and the increase in serum TSH and the depletion of pituitary TSH were subnormal (Bakke and Lawrence, 1966). These rats were also shown to have a subnormal response to thyrotropin-releasing hormone (TRH) stimulation, and assay of hypothalamic tissue indicated significantly increased concentrations of TRH (Bakke, Lawrence, and Wilber, 1974). We have called this group of abnormalities the "neo-T4 syndrome."

It was also demonstrated that the implantation of T4 in systemically in-

effective doses into the arcuate area of the hypothalamus of neonatal rats would produce the neo-T4 syndrome (Bakke et al., 1972), suggesting that T4 acted directly on certain developing centers regulating pituitary function. However, the possibility that the T4 may have acted on the anterior pituitary could not be excluded. To test the hypothesis that the neo-T4 syndrome is a hypothalamic disorder, we have conducted further experiments testing the feedback regulation of TSH. Averill, Purves, and Sirett (1961) and subsequently Martin, Boshans, and Reichlin (1970) reported that destruction of the hypothalamic "thyrotropic area" altered the feedback interaction of thyroid hormone so that the pituitary became more sensitive to inhibition, i.e., there was a lowering of the "set point" of hypothalamic-pituitary regulation. We have found that rats with the neo-T4 syndrome respond in a similar way to the hypothalamic-lesioned rats. These experiments are described below.

Materials and Methods

The methods are detailed in the references cited above and will be presented only briefly here. Timed-pregnant Sprague-Dawley rats were obtained by limiting sexual exposure to a selected 24-hr period. Rats born in a given 24-hr period were sorted to make litters of eight to 10 males or females. These rats were maintained on Purina Laboratory Chow and tap water *ad lib* and were kept in quiet quarters with a controlled temperature and diurnal lighting. Treated pups were injected subcutaneously with T4 in alkaline saline daily for 4 to 7 days. The total quantity of injected T4 varied between 135 and 225 μg. Control animals were injected on the same schedule with vehicle alone. Animals were killed by decapitation while lightly anesthesized with sodium phenobarbital, and trunk blood was collected. The individual serum samples were frozen for TSH determinations by radioimmunoassay. All were killed between 1,300 and 1,600 hr to avoid changes arising from circadian variations in TSH secretion (Bakke and Lawrence, 1965). At necropsy the pituitary, thyroid, adrenals, gonads, etc. were immediately weighed on torsion balances. Pituitary glands were homogenized in cold isotonic Krebs-Ringer phosphate buffer and stored in the frozen state until assayed for TSH. In the particular experiments reported here, the rats were allowed to mature, after which time groups were challenged for 10 days with PTU, 0.00625%, in the drinking water. The feedback sensitivity to T4 administration was based on the protocol used by Martin et al. (1970). One group of rats received a daily "small" dose of T4 dissolved in alkaline saline and injected subcutaneously for 10 days. Another group received a larger dose. The control group received daily saline injections. In the initial experiment, using males, the doses of T4 were 0.5 μg/100 g body weight per day (bw/d) and 2.0 μg/100 g bw/d. A subsequent study in females employed 0.7 μg/100 g bw/d and 1.1 μg/100 g bw/d. These dose changes in T4 were selected in an attempt to more sharply delineate the changes in feedback sensitivity.

In another experiment to test the feedback sensitivity of PTU-treated rats to a single dose of T4, 20 μg/100 g bw were injected 24 hr before termination to provoke a rebound rise in pituitary TSH content. This was done according to the method previously reported by Bakke and Lawrence (1964). In this experiment, which antedated the radioimmunoassay, TSH was measured by bioassay (Bakke, Heideman, Lawrence, and Wiberg, 1957), and the results were expressed with 95% confidence limits.

Results

Table 1 summarizes two experiments confirming and extending the observations noted in the introduction. Table 2 and Fig. 1 show measurements of feedback regulation in neo-T4 rats when the circulating thyroid hormone concentration was manipulated. After thyroid hormone synthesis was blocked with PTU for 10 days, the adult, neo-T4 rats showed an impairment in pituitary and thyroid hypertrophy as compared with controls.

It may be seen that PTU administration produced a marked decrease in pituitary TSH content in both groups, and in the case of the females the depletion was significantly greater in the neo-T4 animals than in the controls. Similarly the marked increase in serum TSH due to PTU administration was also present in the neo-T4 animals, but it was significantly less than in their controls. Goiter growth was unaffected in both controls and neo-T4 males receiving T4, 0.5 μg/100 g bw/d. However, the serum TSH concentration was significantly depressed in the neo-T4 animals (Table 2 and Fig. 1). In addition it may be seen that the larger dose of T4 (2 μg/100 g bw/d) suppressed the serum TSH to very low levels and abolished goiter growth; in fact the thyroid weights were less than found in controls, as might be expected from the marked suppression of the serum TSH concentration. Suppression of the serum TSH was so marked that there was no significant difference between the control and the neo-T4 animals. However, the pituitary TSH content in the neo-T4 males was significantly lower than that in controls.

When the females were treated with doses of T4 that were adjusted to more clearly demonstrate the differential sensitivity in feedback regulation, it was observed that a dose of 0.7 μg/100 g bw/d caused a fall in serum TSH from 75.6 ± 5.7 mU/100 ml to 44.9 ± 5.7, whereas in neo-T4 animals this dose of T4 more completely suppressed serum TSH, down to 6.8 ± 3.9 mU/100 ml ($p < 0.001$). The slightly larger dose of 1.1 μg/100 g bw further suppressed the normal females to 14.9 ± 3.4 mU/100 ml, a value still above the baseline level; the neo-T4 animals were suppressed even more markedly to 2.1 ± 0.5 mU/100 ml ($p < 0.001$). The small dose of T4 did not prevent the marked pituitary TSH depletion caused by PTU in control animals,

TABLE 1. *Late effects of neonatal T4 treatment*

	Experiment A				Experiment B			
	Co	Neo-T4	Co	Neo-T4	Co	Neo-T4	Co	Neo-T4
Age (days)	273	273	197	197	124	124	157	157
Sex	Males	Males	Females	Females	Males	Males	Females	Females
Final N	15	15	19	24	5	5	5	5
Eye-opening (days)	15.5	11.2**	15.5	11.2**	14.9	10.3**	14.8	10.1**
Wean wt (g)	47.1	38.4**	47.0	39.0**	43.2	41.0	41.7	47.6**
Final wt (g)	617	446**	334	259**	487	387**	307	260**
Vag. open (days)	—	—	34.9	39.2**	—	—	34.7	37.7+**
1st Estrus (days)	—	—	35.4	41.8**	—	—	34.7	37.7+**
Est. cycle (days) min	—	—	3.85	5.02**	—	—	—	—
max	—	—	4.91	5.96**	—	—	—	—
Pit. wt (mg)	15.0	9.61R**	17.4	11.7**RR	13.1	9.62	15.5	12.0**
Thy. wt (mg)	22.1	17.2**	17.0	12.3	19.0	14.8**	15.5	11.2**
Adr. wt (mg)	48.5	37.6**	69.0	51.5**	52.0	41.6*	65.9	47.6**R
Gon. wt (g, mg)	3.78	2.14**RR	107	77.7**	3.82	2.15**RR	106	84.6
Pros. wt (mg)	741	680R	—	—	786	413RR	—	—
Uter. wt (mg)	—	—	574	517	—	—	565	451
Pit. TSH (mU)	326	168**	137	95.2*	304	146	288	223
SME-TSH (μU)	—	—	—	—	330	501	—	—
Serum TSH (μU/ml)	49.2	27.0**	20.8	16.5**	125	78.5	109	91.5
Serum LH (ng/ml)	2.2	2.3	—	—	—	—	—	—
Serum FSH (ng/ml)	613	651	—	—	—	—	—	—
Serum Prolactin (ng/ml)	60.0	56.3	—	—	—	—	—	—
SME-TRH (ng)[b]	5.58	7.12**	—	—	—	—	—	—
PTU response:[a]								
Pit. TSH (mU)	—	—	—	—	60	40	59	39**
Serum TSH (μU/ml)	—	—	—	—	590	261**	756	554*
Thy. wt (mg)	—	—	—	—	45.3	26.6*	41.4	29.9**
Serum TRH (pg/ml)[b]	101.1	57.7*	—	—	—	—	—	—

[a] PTU 0.00625% drinking water for 10 days.
[b] TRH Assays by Dr. John Wilber; gonadotropin assays by C. Y. Bowers.
* indicates $p < 0.05$; **, $p < 0.01$.
R and RR indicate significance when relative weights were used.

TABLE 2. *Effect of PTU challenge and T4 replacement in neo-T4 rats*

			PTU[a] + Saline		PTU + T4 (small dose[b])		PTU + T4 (larger dose[c])	
	N[d]	Sex	Control	Neo-T4	Control	Neo-T4	Control	Neo-T4
BW wt	5–6	F	316 ± 11	258 ± 6**	326 ± 8	256 ± 12**	314 ± 7	253 ± 10**
(g)	6–8	M	454 ± 8	338 ± 23**	495 ± 12	342 ± 21**	474 ± 11	342 ± 18**
Pit. wt	5–6	F	16.88 ± 0.72	12.39 ± 0.89**	17.64 ± 0.76	11.78 ± 0.58**R	17.15 ± 0.85	12.66 ± 0.32**
(mg)	6–8	M	12.56 ± 0.58	8.86 ± 0.83**	13.32 ± 0.55	9.17 ± 0.54**	13.13 ± 0.57	8.45 ± 0.65**
Thy. wt	5–6	F	41.4 ± 3.2	29.9 ± 0.9**	38.4 ± 2.2	14.2 ± 1.9**RR	31.3 ± 2.9	12.0 ± 0.8**
(mg)	5–8	M	45.3 ± 3.0	26.6 ± 6.2**	45.6 ± 2.7	24.7 ± 3.7**	15.6 ± 0.7	11.2 ± 1.3*
Pit. TSH	5–6	F	59.1 ± 5.6	39.1 ± 3.2**	91.9 ± 6.3	194 ± 38*	270 ± 55	191 ± 29
(mU/pit)	5–8	M	60.0 ± 5.2	40.2 ± 8.2	73.2 ± 8.9	88 ± 21	304 ± 81	120 ± 30*
SERUM TSH	5–6	F	75.6 ± 5.7	55.4 ± 5.4*	44.9 ± 5.7	6.84 ± 3.9**	14.9 ± 3.4	2.17 ± 0.5**
(mU/100 ml)	5–8	M	59.0 ± 3.9	26.1 ± 4.9**	47.4 ± 10.2	17.3 ± 3.0*	1.36 ± 0.2	0.97 ± 0.2

[a] PTU, 0.00625%, in drinking water for 10 days. These rats also received daily saline injections to be comparable to the two groups injected with T4.
[b] T4, injected daily for 10 days. Small dose was 0.5 μg/100 g bw in males; 0.7 μg/100 g bw in females.
[c] Larger dose of T4 was 2.0 μg/100 g bw in males; 1.1 μg/100 g bw in females.
[d] N indicates number in the smallest and largest group.
Astericks and R are the same as in Table 1. All comparisons are between the control and neo-T4 groups.

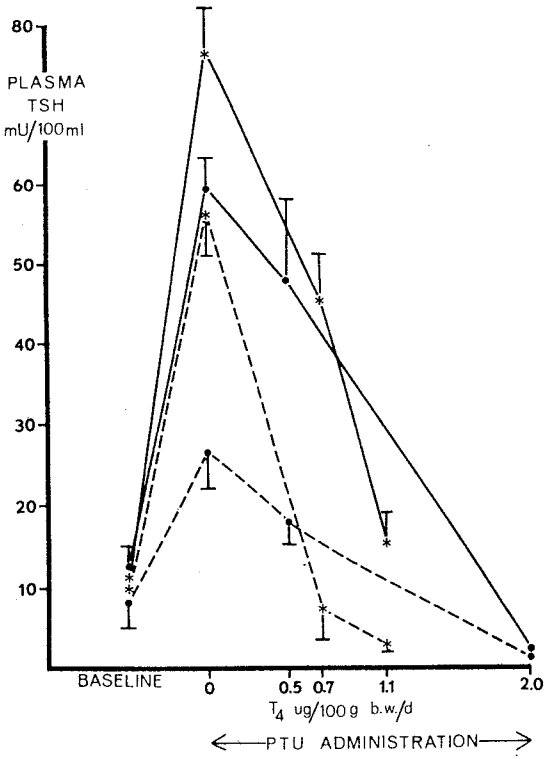

FIG. 1. Baseline values were obtained just before starting PTU drinking water, 0.00625%, for 10 days. The daily dose of injected T4 is indicated. Each point is a mean of six to eight animals ± SE. The neo-T4 rats show a significant hypersensitivity to the suppressive action of T4 on TSH secretion. (●) Male; (*) female; (—) control; (--) neo-T4.

but it did prevent this depletion in the neo-T4 group. The thyroid weights also showed greater sensitivity to T4 suppression in the neo-T4 rats. Neither the small nor large dose of T4 significantly suppressed goiter growth in controls. However, the small dose of T4 in neo-T4 animals kept the thyroid weight down so that it did not differ significantly from the baseline value and the larger dose abolished all goiter growth.

Pituitary weight was not significantly affected by T4 administration in any of the groups, but in every instance the pituitary weight was significantly lower in the neo-T4 rats as compared with controls. Similarly the thyroid weight was significantly lower in the neo-T4 rats in every group.

A rebound phenomenon is seen when a single dose of T4 is given to PTU-treated rats; TSH secretion is inhibited and pituitary TSH content increases sharply, sometimes as much as eightfold (Bakke and Lawrence, 1964). Table 3 shows the results of such an experiment in control and neo-T4-

TABLE 3. *Effects of Neo-T4 treatment on adult pituitary TSH depletion and net TSH synthesis*[a]

	Baseline[b]	PTU Challenge[c]	+T4 Blockade[d]
Control (neo-saline)			
Pit. TSH (mU/pit)	150(121–185)	25.2(20.2–31.5)	272(215–343)
Net Change (mU/pit)	—	−125(101–153)	+247(195–312)
Neo-T4 treated			
Pit. TSH (mU/pit)	88.0(72.6–107)	22.5(18.7–27.0)	105(87.5–125)
Net Change (mU/pit)	—	−65.6(53.9–79.6)	+82.1(68.7–98.0)

[a] 36 male rats, 18 neo-T4 and 18 saline controls were killed at 115 days of age; six rats per each subgroup. Brackets show 95% confidence limits.

[b] Values obtained when groups were killed at 115 days of age.

[c] Values obtained after 7 days of treatment with PTU (0.00625% in drinking water) started when 108 days old.

[d] Values obtained 24 hr after injecting T4 (20 μg/100 g body wt) into rats that had received PTU for 7 days starting at 107 days of age. The increase represents net pituitary TSH synthesis over the 24-hr period.

treated rats. In this experiment a PTU challenge of 7 days duration caused a decrease in pituitary TSH of 125 mU in the controls and 65.5 mU in the neo-T4 rats. The control rats showed an increase in net TSH synthesis of 247 (195 to 312) mU 24 hr after a single T4 injection, whereas the neo-T4 animals had an increment of only 82.1 (68.7 to 98.0) mU per gland.

Discussion

The results of these experiments show that neo-T4 rats qualitatively respond like rats with bilateral anterior hypothalamic lesions in the thyrotropic area. Martin et al. (1970) reported that in lesioned rats the plasma TSH was often reduced to undetectable levels and following reduction of the thyroid hormone concentration by either PTU treatment or thyroidectomy the TSH levels increased more slowly than in controls and did not achieve the concentration seen in the intact controls. When thyroidectomized rats were treated with T4 by daily injection of a dose of 0.5 μg/100 g bw, plasma TSH was effectively suppressed in lesioned rats, whereas there was no measurable effect in intact rats. In addition the lesioned rats failed to show the normal hypertrophy of the pituitary and depletion of pituitary TSH following thyroidectomy. They concluded that this observation suggested that hypothalamic lesions increased the sensitivity of the pituitary to T4 inhibition of TSH synthesis as well as release. They also found that the control thyroidectomized rats showed a progressive repletion of pituitary TSH content with increasing doses of T4, whereas the lesioned animals showed a biphasic response, the small dose of T4 producing an increase and the large dose a depression in pituitary TSH content. They suggested that this may be related to the rebound phenomenon reported by D'Angelo (1969) and Bakke and Lawrence (1964).

Martin et al. (1970) did not perform the rebound experiments using a single dose of T4 in the hypothyroid-lesioned rat. As shown in Table 3, when TSH secretion was inhibited by T4, the net increase in pituitary TSH was significantly greater in the control rats than in the neo-T4 rats, suggesting an impairment in TSH synthesis. When T4 was given daily with PTU (Table 2), pituitary TSH depletion from PTU administration was significantly reversed by a small dose of T4, 0.7 μg/100 g bw/d. The mean pituitary TSH was higher in neo-T4 females than in control females (194 $\pm$ 38 versus 92 $\pm$ 6.3 mU/gland, $p < 0.05$). The larger doses of T4 completely prevented the depletion of pituitary TSH in the control animals, whereas in the neo-T4 males the larger dose caused a significant decline in pituitary TSH content similar to the biphasic response reported by Martin et al. (1970). These results suggest that in the neo-T4 rat, and probably in the lesioned rat as well, there is increased sensitivity of the pituitary to T4 inhibition of TSH synthesis and release.

Thus these observations support the theory that neonatal thyrotoxicosis results in a persistent alteration of hypothalamic function with an abnormal sensitivity to feedback regulation of TSH synthesis and secretion by circulating thyroid hormone levels and that the abnormality is similar to that seen in rats with specific hypothalamic lesions. An alteration in pituitary function is not excluded by these experiments and, if present, might result from a persistent deficiency of tonic TRH stimulation from the hypothalamus. This is supported by the recent measurements of serum TRH just completed in collaboration with Dr. John Wilber. Control rats had a mean serum concentration of 101.1 $\pm$ 15.1 pg/ml TRH, whereas neo-T4 rats had a significantly lower concentration (57.7 $\pm$ 9.4 pg/ml, $p < 0.05$). Thus the increased SME content of TRH in the neo-T4 syndrome may represent a "back-up" pooling secondary to impaired secretion analogous to the rebound increase in pituitary TSH content when secretion is blocked with thyroid hormone. It is further suggested that this chronic TRH deficiency results in blunting of pituitary thyrotrophe responsiveness and a decreased response to TRH stimulation as observed in neo-T4 rats.

II. LATE EFFECTS OF PERINATAL HYPOTHYROIDISM ON CERTAIN ENDOCRINE REGULATORY SYSTEMS IN THE ADULT RAT

We were the first to report the consequences of a brief episode of hypothyroidism during the critical perinatal period of development (Bakke, Gellert, and Lawrence, 1970). We found that PTU treatment during the perinatal period (PTU in maternal drinking water the last 5 days of gestation or injected into pups in gum acacia during the first 4 days of life) caused a persistent increase in thyroid weight and elevated pituitary TSH content. These changes persisted through adult life and were observed after as few

as two injections of PTU in the neonatal period. Although we were unable to measure serum TSH concentrations in these early experiments, the goiters in neo-PTU rats appeared histologically inactive, and the circulating protein-bound iodine (PBI) and T4 levels were reduced. As adults there was evidence of impaired TSH synthesis when challenged with PTU. We observed an increase in the SME TSH in neo-PTU rats, although the significance of the observation has not been elucidated (Bakke and Lawrence, 1967). We also reported that the perinatal PTU treatment resulted in delayed puberty as indicated by late vaginal opening and prolonged estrous cycles. The gonadal alterations produced by prenatal PTU treatment were not corrected by T4 replacement during the neonatal period, although the pituitary-thyroid and SME-TSH abnormalities were corrected by this therapy. Thus late thyroidal effects depended upon neonatal rather than prenatal hypothyroidism. The late effect of both the neo-T4 and neo-PTU syndromes seemed to be persistent mild hypothyroidism, even though it was produced by different mechanisms.

Further Studies

More recently we have completed additional experiments, two of which are summarized in Table 4. It may be seen that eye-opening in neo-PTU rats is significantly delayed and weaning weights are significantly diminished, although the final weights were not significantly affected. Thyroid weights were increased both absolutely and relatively. Pituitary weights were significantly diminished both absolutely and relatively in both sexes. Adrenal weights were diminished in the males but were increased significantly in the females. The testes were significantly increased in weight, but the ovaries were not. The serum TSH concentrations showed small differences, but these were not usually statistically significant in a given experiment. In a total of seven experiments, pituitary TSH was increased and serum TSH was elevated with the single exception shown in Table 4 (Experiment A, males).

The response to TRH stimulation was significantly blunted in neo-PTU rats (Fig. 2). The metabolic clearance of injected labeled TSH was similar in control and neo-PTU rats. Serum TRH assays performed by Dr. John Wilber (Table 4) showed no significant alteration in neo-PTU rats, unlike the depressed values in the neo-T4 syndrome.

In one experiment we have obtained measurements of serum LH, FSH, and prolactin (through the generosity of Dr. C. Bowers). The only abnormality was a significant elevation in serum prolactin. Serum T4 was measured by the Murphy-Pattee method in individual rats and was found to be diminished in neo-PTU rats (Table 4) confirming earlier experiments (Bakke et al., 1970).

TABLE 4. *Late effects of perinatal PTU treatment*

	Experiment A[a]				Experiment B[b]			
	Co	PTU	Co	PTU	Co	PTU	Co	PTU
Age (days)	147	147	258	258	171	171	178	178
Sex	Males	Males	Females	Females	Males	Males	Females	Females
Final N	29	29	17	16	10	10	10	10
Eye-opening (days)	15.6	16.0*	15.3	16.6**	14.3	17.4**	14.1	17.2**
Wean wt (g)	42.8	42.6	43.5	36.4**	42.5	34.9**	43.7	31.8**
Final wt (g)	546	531	336	336	538	497	282	296
Vag. open (days)	—	—	33.2	38.2**	—	—	35.4	40+
1st Estrus (days)	—	—	34.7	39.6**	—	—	35.7	40+
Est. cycles (days) min	—	—	3.22	3.42*	—	—	—	—
max	—	—	4.15	4.32	—	—	—	—
Pit. wt (mg)	13.2	12.3	18.0	16.1	14.2	10.9**RR	20.6	12.5**RR
Thy. wt (mg)	19.6	23.2**RR	18.3	17.7	19.9	21.8RR	13.7	16.6*
Adr. wt (mg)	45.4	44.4	60.1	57.9	50.4	44.5**	53.1	67.4*R
Gon. wt (g, mg)	3.72	3.74	78.4	68.7R	3.76	4.22*RR	61.6	74.6
Pros. wt (mg)	658	539**RR	—	—	695	685	—	—
Uter. wt (mg)	—	—	734	588*	—	—	633	629
Pit. TSH (mU)	266	326	205	318*	351	450	315	578**
SME-TSH (μU)	70	120	67	112	—	—	—	—
Serum TSH (μU/ml)	100	59	44	56	86	135	90	120
Serum T4 (μg%)	7.00	5.44**	—	—	—	—	—	—
Serum LH (ng/ml)	1.48	1.28	—	—	—	—	—	—
Serum FSH (ng/ml)	238	249	—	—	—	—	—	—
Serum Prolactin (ng/ml)	30	47*	—	—	—	—	—	—
PTU response[c]:								
Pit. TSH	—	—	87	92	—	—	—	—
Serum TSH	—	—	156	222	—	—	—	—
Thy. wt	—	—	31.4	34.8	—	—	—	—
Serum TRH (pg/ml)	—	—	—	—	63	46	70	68

[a] PTU 0.05% drinking water from the 17th day of gestation to 5th day post partum and pups injected with 2 mg PTU in gum acacia daily for 5 days. Controls received saline injections.

[b] PTU 0.025% drinking water from the 18th day of gestation to birth then 0.00625% to 5th day post partum. Pups injected as in [a].

[c] PTU 0.00625% drinking water for 7 days.

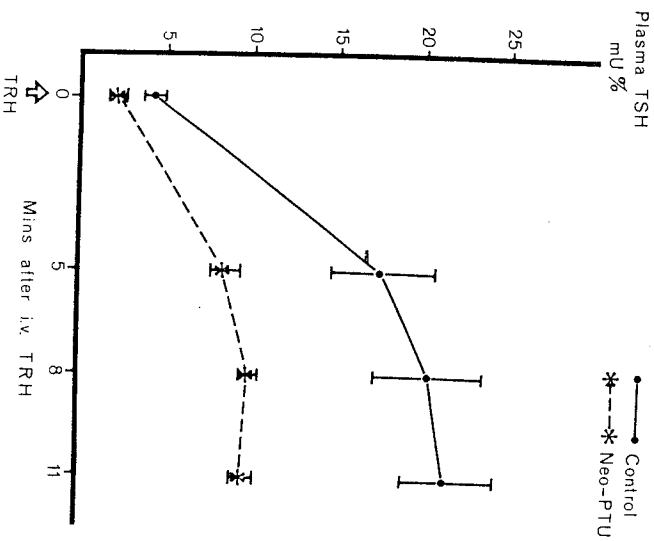

FIG. 2. Plasma TSH response in neo-PTU male rats following the iv injection of TRH, 200 ng/100 g bw. Each point is the mean of five samples withdrawn via caval catheter. Standard errors are shown. The blunted response in the neo-PTU rats is significant, $p < 0.002$.

Discussion

Table 7 shows an overall summary comparing the neo-PTU syndrome with other neonatal syndromes. There are interesting similarities to the neo-T4 syndrome. Adults from both groups have low serum T4 levels and an impaired response to TRH. Both have delayed puberty, prolonged estrous cycles, and marked reductions in body and pituitary weight. However, there are striking differences between the two groups as regards thyroid weight, gonadal weight, pituitary and serum TSH concentrations, and in the response to PTU challenge. It may be theorized that the hypothalamic set point for TSH regulation has been altered in the neo-PTU syndrome but direct evidence for this is presently lacking.

III. LATE EFFECTS OF NEONATAL UNDERNUTRITION AND OVERNUTRITION ON ADULT ENDOCRINE FUNCTION

It has been established that when rats are undernourished during the neonatal period and then allowed free access to food there are irreversible

life-long effects on body size (Kennedy, 1957; Knittle and Hirsch, 1968; Widdowson and Kennedy, 1962; Widdowson and McCance, 1962; Winick, 1971; Winick and Noble, 1966; Bakke, Lawrence, and Bennett, 1973), on brain DNA (Schain and Watanabe, 1973; Schain, Watanabe, and Harel, 1973), on fat cell number and size (Knittle and Hirsch, 1968; Winick and Noble, 1966; Winick, 1971), on accumulation of catecholamines in the brain (Shoemaker and Wurtman, 1971), on cardiac and respiratory rates (Hofer, 1973), and on behavior (Hofer, 1973; Schain et al., 1973; Simonson, Sherwin, Anilane, Yu, and Chow, 1969). These effects do not follow starvation of adult rats. The mechanism by which undernutrition during the neonatal period produces these effects is not clear. It has been shown that maternal dietary restriction during gestation and lactation results in progeny with smaller pituitary glands containing reduced concentrations of growth hormone (Stephan, Chow, Frohman, and Chow, 1971) and reduced levels of plasma growth hormone (Sinha, Wilkins, Selby, and Vanderlaan, 1973). Moreover Shambaugh and Wilber (1974) have shown that neonatal undernutrition results in hypothyroidism during the period of the nutritional deprivation and that this is due to hypothalamic TRH deficiency. These observations suggest that the persistent hypothyroidism following the neo-T4 syndrome may be secondary to relative caloric deprivation rather than a direct effect of T4. Thus we conducted studies of the long-term consequences of neonatal undernutrition as compared with both overnutrition and normal nutrition.

Materials and Methods

Rats born in a given 24-hr period were sorted to make up litters of 15, 8, and 3 to provide a comparison between underfed and overfed with "normal" litters of eight. When the rats were weaned at 21 days of age, they were housed two to a cage and provided with food and water *ad lib* for the remainder of the experiment. Other details are as previously stated.

Results

Table 5 shows that at 21 days of age the underfed rats were significantly smaller, and the overfed rats were significantly larger than controls. These rats were allowed to mature and were killed at 120 days of age, at which time the underfed rats were still significantly smaller; the overfed rats did not differ from controls. Eye-opening was significantly delayed in the underfed females but vaginal opening, first estrus, and estrous cycle length were not altered. Pituitary weights (mg/100 g bw) were significantly larger in the underfed females. The difference was not significant in males. Adrenal weights also were significantly increased in both the underfed and overfed females but not in males. Testes and uterine weights were significantly

TABLE 5. *Late effects of neonatal under- and overfeeding*[a]

	Sex	n	Neounderfed	Control	Neooverfed
BW (wean) (g)	F	28–30	31 ± 1**	48 ± 1	55 ± 1**
	M	28–30	32 ± 1**	49 ± 1	52 ± 1
BW (final) (g)	F	19–20	268 ± 4**	295 ± 7	285 ± 5
	M	19–20	428 ± 7**	475 ± 7	490 ± 6
Eye-opening (days)	F	28–30	15.7 ± 0.1**	14.8 ± 0.2	14.7 ± 0.2
	M	28–30	15.3 ± 0.1	14.9 ± 0.2	14.7 ± 0.2
Vag. open (days)	F	27–30	34.4 ± 0.4	34.6 ± 0.5	34.6 ± 0.5
1st Estrus (days)	F	27–30	34.7 ± 0.4	34.8 ± 0.5	34.6 ± 0.4
Est. cycle length (days)	F	27–30	3.28 ± 0.1	3.41 ± 0.1	3.33 ± 0.1
Pit. wt (mg/100 g)	F	19–20	6.16 ± 0.13**	5.53 ± 0.15	5.42 ± 0.11
	M	19–20	2.82 ± 0.06	2.68 ± 0.04	2.65 ± 0.05
Thy. wt (mg/100 g)	F	19–20	5.9 ± 0.2	5.7 ± 0.2	5.5 ± 0.2
	M	19–20	4.6 ± 0.2	4.1 ± 0.2	3.8 ± 0.1
Adr. wt (mg/100 g)	F	9–10	24.5 ± 0.5**	20.7 ± 0.9	24.7 ± 1.1*
	M	14–15	11.3 ± 0.3	10.8 ± 0.6	10.2 ± 0.4
Ovary wt (mg/100 g)	F	19–20	30.4 ± 2.0	29.8 ± 1.0	29.8 ± 1.4
Testes wt (g/100 g)	M	19–20	0.83 ± 0.02*	0.77 ± 0.01	0.88 ± 0.74**
Uter. wt (mg/100 g)	F	9–10	201 ± 16**	149 ± 7	184 ± 14**
Vent. pros. wt (mg/100 g)	M	14–15	120 ± 4	116 ± 8	108 ± 5
SME-TSH (mU/SME)	F	7–9	0.10 ± 0.01	0.19 ± 0.04	0.22 ± 0.01
	M	9–10	0.13 ± 0.03	0.17 ± 0.02	0.21 ± 0.05
Pit. TSH (mU/pit)	F	9–10	275 ± 35	237 ± 16	279 ± 25
	M	9–10	286 ± 23	312 ± 42	294 ± 25
Serum TSH (mU/100 ml)	F	9–10	7.0 ± 1.1*	3.5 ± 0.6	5.3 ± 1.2
	M	9–10	12.4 ± 3.3	10.9 ± 2.9	11.7 ± 2.7
Serum T4 (μg/100 ml)	F	5–10	3.85 ± 0.35	4.02 ± 0.28	3.32 ± 0.32
	M	5–10	4.45 ± 0.32	4.91 ± 0.40	5.98 ± 0.19

[a] The rats underfed in the neonatal period were raised in litters of 15, the controls in litters of 8, and the overfed in litters of 3. n Indicates the smallest and the largest group for the designated line of data. Only relative organ weights are given. The females were killed when 118 to 127 days of age and the males when 111 to 120 days of age. Significance designations indicate differences from the control group. Asterisks same as in Table 1.

larger in both the underfed and overfed groups. SME and the pituitary gland TSH contents were not significantly altered in any of the groups but the serum TSH was significantly elevated in the underfed females (7.0 mU/100 ml compared with 3.5); no differences were noted in males. Serum T4 values were lower in both underfed males and females but the difference was not statistically significant. Overfed males had a higher serum T4 than either the control or underfed groups, but the overfed females had lower values. None of these differences was significant.

Table 6 shows the results of a 7-day challenge with PTU in the drinking water when the rats were adults. There was no significant difference between the groups with regard to pituitary weight, thyroid growth, or adrenal weight;

TABLE 6. *Result of PTU challenge in neonatally under- and overfed rats*[a]

	Sex	n	Neounderfed	Control	Neooverfed
Pit. wt (mg/100 g)	F	9–10	6.41 ± 0.22	5.73 ± 0.27	5.38 ± 0.24
	M	9–10	2.81 ± 0.11	2.56 ± 0.13	2.66 ± 0.12
Thy. wt (mg/100 g)	F	9–10	12.6 ± 0.7	12.0 ± 0.5	12.7 ± 0.5
	M	9–10	9.3 ± 0.3	7.2 ± 0.5	7.9 ± 0.3
Adr. wt (mg/100 g)	F	9–10	23.0 ± 0.9	21.4 ± 1.2	22.7 ± 0.9
	M	9–10	10.3 ± 0.4	9.2 ± 0.3	9.4 ± 0.2
Ovary wt (mg/100 g)	F	9–10	29.5 ± 1.1**	34.4 ± 1.0	30.9 ± 1.6
Testes wt (g/100 g)	M	9–10	0.75 ± 0.02	0.74 + 0.03	0.74 ± 0.02
Uter. wt versus (mg/100 g)	F	9–10	183 ± 13	205 ± 19	181 ± 12
Pros. wt (mg/100 g)	M	9–10	120 ± 7	103 ± 6	113 ± 4
SME-TSH (mU/SME)	F	7–8	0.15 ± 0.02	0.12 ± 0.02	0.16 ± 0.04
	M	9–10	0.33 ± 0.07	0.21 ± 0.04	0.34 ± 0.07
Pit. TSH (mU/pit)	F	9–10	50 ± 4	49 ± 6	53 ± 11
	M	9–10	31 ± 2**	59 ± 10	32 ± 3*
Serum TSH (mU/100 ml)	F	9–10	43.3 ± 4.6	36.5 ± 5.4	41.4 ± 4.3
	M	9–10	39.0 ± 4.1	33.8 ± 2.5	33.2 ± 2.0

[a] These are rats taken from the same experiment shown in Table 5 and placed on PTU, 0.00625% in the drinking water for 7 days when they were adult. The effects of the PTU challenge may be ascertained by comparing the data with those shown on Table 5. The asterisks here indicate a significant difference from the control group, which of course received the same PTU challenge.

however, ovarian weight was significantly less in the underfed group and pituitary TSH content was significantly reduced in the males of both the neonatally underfed and overfed groups. The marked increase in serum TSH was similar in the three groups. PTU produced a significant increase in thyroid weight and serum TSH and a decline in pituitary TSH in all groups. In addition, there was a significant increase in relative ovarian and uterine weights in control but not in treated animals. The only other significant effect of PTU was a decrease in testicular weight both absolutely and relatively in the neonatally overfed group.

Figure 3 shows the results of TRH administration. The response appears to be normal in all groups and both sexes. The apparent greater increase in serum TSH at 5 min in the underfed group does not differ significantly from the other groups.

It is of interest that males had consistently higher serum TSH concentrations than females. When the serum data shown in Table 5 are combined according to sex, the difference becomes highly significant ($p < 0.001$). In spite of this, the thyroid, pituitary, and adrenal weights were significantly lower in the males. Males also demonstrated a greater increase in serum TSH after TRH stimulation than females. In other experiments (not shown) using groups of 10 animals, males had higher TSH concentrations after TRH than females at 8 min (51.5 ± 5.3 mU/100 ml versus females, 21.8 ± 2.7, $p < 0.001$) and 15 min (54.7 ± 7.7 mU/100 ml versus females, 27.5 ± 4.3, $p < 0.02$).

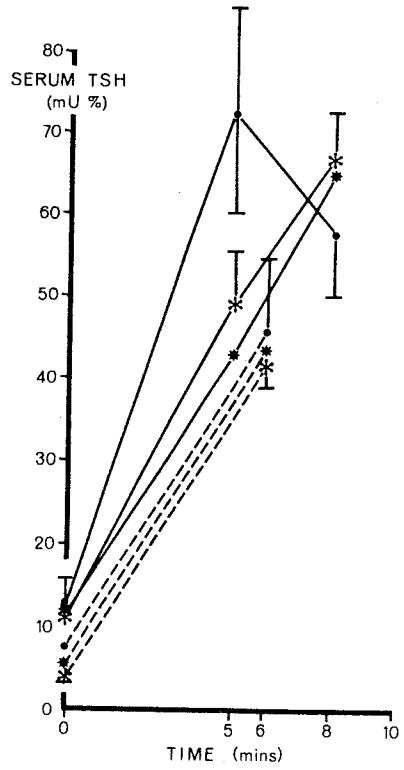

FIG. 3. Each point is the mean of five rats 120 days of age killed at the indicated times following the iv injection of TRH, 50 ng/100 g bw. The mean and standard errors are shown. The under- and overfed groups did not differ significantly from the controls. The females had significantly lower TSH concentration than the males both before and after TRH. (*) Control; (●) neo-underfed; (•) neo-overfed; (—) males: (– –) females.

Discussion

These results confirm that neonatal underfeeding of rats results in a lifelong decrease in body size. Although this has been reported many times, this effect was not observed by Azizi, Vagenakis, Bollinger, Reichlin, Braverman, and Ingbar (1974) and Sinha et al. (1973). The latter authors reported catch-up growth after only 2 weeks of free access to food, and this catch-up was associated with a striking elevation in plasma growth-hormone concentrations.

Perhaps as suggested by Schain et al. (1973), using rabbits, animals manifesting catch-up growth are somehow able to resume more vigorous feeding when presented with adequate food. Acidosis and hypoglycemia may have weakened some pups more than others, so that reduced intake persisted for a longer time.

It is of some interest that our group of overfed rats, although much heavier when weaned, is not heavier as adults, in contrast to the results of Knittle and Hirsch (1968). These authors, however, compared only two groups of animals; the undernourished animals were raised in litters of 22, whereas

the overnourished were in litters of 4. In addition, there were no normal controls in their experiments, so their results could be misleading.

There was a positive correlation between serum T4 and body weight in the present studies (Table 5) which is not explained. Stabenau and Pollin (1968), studying 23 pairs of adult monozygotic twins, showed a significant correlation between birth weight (which is more comparable to rat weaning weight than birth weight) and adult PBI. These authors suggested that genetic similarity may not be as important in determining the PBI as individual nongenetic differences related to intrauterine factors. Perhaps these are comparable to neonatal nutritional factors in the rat.

The contrast between neonatally undernourished and neo-T4 rats is of interest because during the hyperthyroidism there is relative undernutrition and failure to gain weight (Table 7). We have previously reported that the delayed puberty in neo-T4 rats does not occur after neonatal food restriction (Gellert et al., 1971). In addition, neo-T4 rats have accelerated eye-opening (Bakke et al., 1972), whereas the neonatally underfed rats have delayed eye-opening (Table 5). The neo-T4 rats have smaller pituitary

TABLE 7. *Comparison of neonatal syndromes*[a]

	Neo-T4	Neo-PTU	Neo-Underfed	Neo-Overfed
Eye-opening	Early**	Late*	Late**	N
Wean wt	Decr*	Decr*	Decr**	Incr** (F)
Final wt	Decr**	N	Decr**	N
Vag. Open	Late**	Late*	N	N
Est. cycles	Longer*	Longer*	N	N
Pit. wt	$Decr^{**RR}$	$Decr^{**RR}$	$Incr^{RR}$ (F)	N
Thy. wt	$Decr^{**R}$	$Incr^{*RR}$	N	N
Adr. wt	$Decr^{**R}$	$Decr^{**}$ (M) $Incr^{**R}$ (F)	$Incr^{RR}$ (F)	$Incr^{R}$ (F)
Gon. wt	$Decr^{*RR}$	$Incr^{*R}$ (M) or N	$Incr^{R}$ (M)	$Incr^{*R}$ (M)
Pros. wt	$Decr^{**RR}$	$Decr^{**RR}$ or N	N	N
Uter. wt	N	Decr* or N	$Incr^{RR}$	$Incr^{RR}$
Pit. TSH	Decr*	Incr*	N	N
SME-TSH	Incr	Decr* or N	N	N
Serum TSH	Decr*	Incr* (M)	Incr* (F)	N
Serum T4	Decr*	Decr*	N	N
TSH half-life	N	N	?	?
TRH response	Decr*	Decr* (M)	N	N
SME-TRH	Incr*	?	?	?
Serum TRH	Decr*	N	?	?
PTU response				
Pit. TSH depl.	Decr*	Incr*	Incr** (M)	Incr* (M)
Serum TSH inc.	Decr*	Incr*	N	N
Goiter growth	Decr*	N	N	Incr

[a] A representative summary of numerous experiments.

Significance designations: * $p < 0.05$; **, $p < 0.01$; R and RR indicate significance of relative weight differences (per 100 g bw). N indicates normal, i.e., differ by less than 10% and not significant. Incr. or Decr. indicates a difference of over 10%, but not significant. (M) indicates in males only; (F), females only.

glands (Bakke et al., 1966, 1974; Gellert et al., 1971), whereas the undernourished rats have a relative increase in pituitary weight. Neo-T4 rats usually have smaller thyroid glands both absolutely and relatively (Bakke et al., 1966, 1974; Gellert et al., 1971); undernourished animals have normal thyroid weights. Neo-T4 rats have significantly smaller adrenal glands, testes, and ventral prostates (Bakke et al., 1974); undernourished rats have significantly larger adrenal glands and larger testes. Neo-T4 rats have normal uterine weights, whereas the underfed rats have significantly increased uterine weights. These observations are in disagreement with the conclusions of Winick and Noble (1966) and Winick (1971) that neonatal feeding results in a diminished number of cells in all tissues. These authors did not study endocrine tissues, which seem to be spared.

The pituitary TSH content and serum TSH, PBI, and free-T4 levels are significantly reduced in neo-T4 rats (Bakke et al., 1966, 1974; Gellert et al., 1971), whereas in neonatally underfed rats the pituitary TSH is normal and the serum TSH tends to be increased. Azizi et al. (1974) also found a small increase in serum TSH in rats that had been calorically deprived neonatally. If this tendency to increased serum TSH levels reflects primary hypothyroidism, one would anticipate an augmented response to TRH stimulation which was not found in our studies (Fig. 3) or those of Azizi et al. (1974). Rather the neo-T4 rat has a diminished response to TRH (Bakke et al., 1974) and to PTU challenge (Bakke and Lawrence, 1966), as indicated both by impaired goiter growth and a blunted serum TSH response (Bakke et al., 1974). By contrast the neonatally underfed and overfed groups responded normally to PTU challenge. The observation that PTU evoked a greater depletion of pituitary TSH in both neonatally underfed and overfed male rats (Table 6) is unexplained. This difference was not reflected in the serum TSH concentrations.

Severely malnourished children have been reported to have either a normal or decreased plasma TSH in one study (Godard and Lemarchand-Beraud, 1973), although another group reported elevated TSH with an exaggerated and sustained response to TRH stimulation (Pimstone, Becker, and Hendricks, 1973). Of course, these studies were made in the presence of malnutrition and may not be comparable to studies of the late consequences of infantile malnutrition. Shambaugh and Wilber (1974) observed hypothalamic TRH deficiency and hypothyroidism in neonatal rats during caloric deprivation. Since we found no indication of hypothyroidism in adult rats that had been calorically deprived, it would appear that the phenomenon they observed at 16 days of age was subsequently reversed when the rats were placed on a normal diet and allowed to mature.

Thus although neonatal caloric deprivation may reduce TRH, TSH, and growth hormone secretion during the period of deprivation, there is no evidence that these abnormalities persist after the rats mature; the persistent reduction in body weight does not appear to be due to any abnormality of

pituitary-thyroid function in the adult. Certainly the abnormalities in the neo-T4 syndrome are not similar to those following neonatal food deprivation.

IV. STUDIES ON THE UNTREATED PROGENY (F_1) OF NEO-T4 MOTHERS

Background

During early studies of neo-T4 rats, we found a significant decrease in fertility in three separate experiments involving large numbers of rats (Bakke and Lawrence, 1968). The 961 pups from these studies (the F_1 progeny) were killed at 5 days of age and their pituitary and SME tissues were assayed for TSH. In each instance it was found that the pituitary TSH was significantly diminished and the SME-TSH was significantly increased. These changes were similar to those in the adult neo-T4 mothers themselves. These unexpected findings prompted us to repeat these experiments and to permit the untreated offspring (F_1) to mature before being tested.

Results

Table 8 shows the results of one of these experiments. Groups of rats of both sexes were killed while still immature (39 and 49 days of age) and remaining groups were allowed to fully mature before killing. The females were rebred to produce the F_2 generation. Thus they were 212 days old when killed. It may be seen that the untreated male offspring of neo-T4 mothers had advanced eye-opening and that the females showed a significant diminution in weaning weight; body weight at 49 days was significantly less than controls. These immature F_1 rats had normal pituitary TSH content but significantly reduced SME TSH; and the serum TSH was significantly increased in both sexes. The females had a significant delay in puberty. When allowed to mature fully, the F_1 males had significantly larger thyroids. Serum TSH was decreased in both sexes, highly significant ($p < 0.01$) in the males. In the immature F_1 females, PTU challenge increased serum TSH to significantly higher concentrations and the goiter weight was significantly lower than in controls. This was not observed in the older animals. Older rats tested with TRH showed a blunted response ($p < 0.01$ in the females).

Since body weight, eye-opening, vaginal opening, first estrus, and thyroid function were abnormal in the F_1 offspring of neo-T4 mothers, we have theorized that these mothers provided an abnormal intrauterine or neonatal environment for their offspring that led to hypothalamic abnormalities. These abnormalities were not as striking as seen in the parents and in some instances were apparent in the offspring when immature but disappeared as the animals matured (i.e., body weight). In the case of serum TSH, there was a biphasic response with aging; the immature F_1 of neo-T4 mothers had an increased TSH concentration, which became decreased when fully mature. Abnormalities also were detected in the untreated F_1 offspring of neo-PTU mothers. These results will not be presented in detail but are summarized in Table 18. It is possible that these effects were due to maternal hypothyroidism so that further studies of this possibility were conducted.

TABLE 8. *Untreated F_1 of controls versus untreated F_1 of neo-T4 mothers and normal fathers*

	Immature F_1				Mature F_1			
	Control F_1 T4 (male)		Control F_1 T4 (female)		Control F_1 T4 (male)		Control F_1 T4 (female)	
Age (days)	39	39	49	49	114	114	212	212
Final N	8	10	10	9	10	10	10	10
Eye-Opening (days)	14.9	14.2**	14.3	14.4	14.9	14.2**	14.3	14.4
Wean wt (g)	51.0	48.1	55.9	45.2**	51.0	48.1	55.9	45.2**
Final wt (g)	151	165	179	151**	474	435	360	326
Vag. Open (days)		—	32.7	34.4**	—	—	32.7	34.4**
1st Estrus (days)		—	33.8	35.3**	—	—	33.8	35.3**
Pit. wt (mg)	5.66	6.09	8.60	8.24R	11.7	11.3	18.7	17.0
Thy. wt (mg)	9.44	10.8	12.3	11.1	14.3	16.9R	15.3	14.7
Adr. wt (mg)	25.8	25.0	48.3	38.2	46.0	48.3	64.7	62.8
Gon. wt (g/mg)	1.65	1.71	58.7	45.6	3.46	3.45	104	92.4
Pros. wt (mg)		—		—	623	540*	—	—
Uter. wt (mg)		—	302	267	—	—	591	594
Pit. TSH (mU)	113	95.0	194	183	266	239	244	269
SME-TSH (μU)	38.0	114**	50.4	26.2**	186	177	51.6	38.9
Serum TSH (μU/ml)	27.0	37.1**	20.5	27.5**	62.5	50.8**	41.6	28.2
5′ *p* TRH		—		—	492	384	399	245**
PTU Response								
Pit. TSH (mU)	30.1	28.7	30.3	39.0	39.5	36.9	43.4	54.8
Serum TSH (μU/ml)	509	551	445	526**	279	255	176	181
Thy. wt (mg)	22.9	19.7	28.2	21.5**R	33.8	35.6	33.6	32.2

* Indicates that the treated group differed significantly ($p < 0.05$) from the control group. R indicates the relative weights differed significantly. Double ** or R indicates $p < 0.01$. All rats were from the same experiment. Some groups were killed when immature (39 and 49 days old) and the remainder when fully mature (111 and 212 days old).

V. CROSS-FOSTERING THE F_1 OFFSPRING OF NEO-T4 MOTHERS

To determine whether the abnormalities in the F_1 offspring of neo-T4 or neo-PTU mothers resulted from abnormalities in lactation and neonatal care or whether they were the result of prenatal influences, normal control pups were placed with neo-T4 mothers at birth until weaned at 21 days. Other cross-fosterings were carried out as outlined in Table 9.

Results and Conclusion

Table 10 shows the results of the four possible combinations of pups and mothers. When control pups were placed with neo-T4 mothers, weaning

TABLE 9. *Analysis of cross-fostering of untreated offspring (F_1)*

Natal parents		Foster		
Mother	Father	Mother	F_1 CODE	N
Co	Co	Co	(Co Co)	48
Neo-T4	Co	Co	(T4 Co)	60
Co	Co	Neo-T4	(Co T4)	58
Neo-T4	Co	Neo-T4	(T4 T4)	60
Co	Neo-T4	Co	(T4 F)	59

Studies on each group (sexes studied separately)
A. Baseline
B. PTU challenge
C. T4 feedback set point
D. TRH response

weights were significantly decreased and eye-opening was delayed in both sexes. Pituitary weight was decreased in the females, thyroid weight increased in both sexes, and there were significant increases in ovarian weight and decreases in prostate and uterine weights. Serum TSH was not significantly altered. It would appear that a neo-T4 foster mother probably was a poor nurser and that this produced the delayed eye-opening and decreased weaning weight of her pups, regardless of whether the F_1 pup was a control or a neo-T4 animal. There is a suggestion that there were more gonadal changes than thyroidal changes when the offspring matured.

It may be seen that when the offspring of neo-T4 mothers were placed with normal foster mothers the weaning weight was restored to normal, eye-opening was no longer delayed, and in the males thyroid weight was significantly increased, possibly reflecting a hypertrophy dependent for its expression on the better nursing provided by normal mothers. However, this was not the case with the females. Here the control pups nursed by neo-T4 mothers had significant goiters (see Sec. VII). It is concluded that some of the abnormalities seen in the untreated F_1 offspring of neo-T4 mothers are the result of neonatal care and others are the result of prenatal influences.

VI. STUDIES OF THE F_2 OFFSPRING OF NEO-T4 MOTHERS

Methods

The untreated F_1 female offspring of control and neo-T4 mothers presented in Sec. IV were allowed to mature to 95 days of age and then mated with normal males. Their F_2 offspring were allowed to mature until 108 days of age when groups of 10 were killed and the usual measurements made.

TABLE 10. *Cross-fostering untreated (F_1) offspring of neo-T4 and control mothers*

	Males				Females			
n	Co Co[a] $n = 8$	T4T4 $n = 10$	Co T4 $n = 8$	T4Co $n = 8$	Co Co $n = 7$	T4T4 $n = 10$	Co T4 $n = 12$	T4 Co $n = 12$
BW wean (g)	46.4	37.8**	38.6**	45.1	43.1	38.7**	34.2**	42.4
BW final (g)	468	448	453	452	248	267	288	283**
Eye-opening age (days)	15.0	15.4*	16.0**	15.3	14.6	15.2*	16.2**	14.9
Pit. wt (mg)	11.38	11.11	11.45	11.35	15.15	13.99^{RR}	15.49^{RR}	14.43^{RR}
Thy. wt (mg)	15.2	17.2	16.8	19.3**^{RR}	13.0	12.6	16.4**	14.0
Adr. wt (mg)	45.0	44.5	44.7	51.3	66.9	61.3^{R}	65.0^{RR}	64.8^{R}
Gon. wt (g/mg)	3.69	3.82	3.47	3.50	81.8	77.9	95.4*^{R}	108.4*^{R}
Pros./uter. (mg)	572	523	418**	561	538	456^{R}	498^{RR}	460^{RR}
Pit. TSH (mU)	246	357	325	327	223	311	326	281
Serum TSH (μU/ml)	50.1	68.7	68.0	58.5	41.7	48.1	37.1	50.6
15′ $\bar{p}$ TRH (μU/ml)	1111	624**	733**	725**	587	739	664	473

[a] For code of groups see Table 9. All rats were killed at 134 days of age. Significances are as presented in Table 7.

Results and Discussion

Table 11 presents the results in verbal form for brevity and simplicity. For purposes of comparison, neo-T4 males and females are again summarized. As in other experiments there were significant differences between the sexes. Some abnormalities present in the parents and the F_1 offspring appeared to fade out in the F_2 offspring, such as the accelerated eye-opening seen in the F_1 males and the decrease in serum TSH concentration in both sexes. On the other hand, there were certain changes, which appeared to have diminished in the F_1 offspring and reappear in the F_2 offspring. For example, the neo-T4 mothers had a significant decrease in pituitary and gonadal weight, which was no longer present in their F_1 progeny but was again present in the F_2 offspring. Both sexes showed a significant increase in thyroid weight in the F_2 offspring in marked contrast to their parents and grandparents. The delay in vaginal opening and first estrus persisted from one generation to the next, being highly significant ($p < 0.01$) in both the F_1 and F_2 offspring of neo-T4 mothers.

Since the F_1 offspring of neo-T4 mothers have certain abnormalities, not so striking as those found in the neo-T4 mothers themselves, but nevertheless differing from controls, one might expect to find abnormalities in the F_2 offspring also. In some cases these abnormalities appear to be more marked in the F_2 than in the F_1 generation, and in other cases the abnormality is in the opposite direction from the parent, i.e., the thyroid atrophy of the parents is replaced by thyroid hypertrophy in the progeny. This difference was much more striking in the F_2 than in the F_1 generation, even though all mothers of F_2 animals were untreated (see Table 12).

TABLE 11. *Comparison of neo-T4 parents with their untreated F_1 and F_2 progeny*[a]

	Males			Females		
	Neo-T4	F_1	F_2	Neo-T4	F_1	F_2
Eye-opening	Early**	Early*	N	Early**	N	N
Wean wt	Decr**	N	N	Decr**	N	N
Final wt	Decr**	Decr	Decr**	Decr**	Decr	N
Pit. wt	Decr**R	N	Incr R	Decr**RR	Decr	Decr*
Thy. wt	Decr**	Incr R	Incr*RR	Decr**	Decr	Incr*RR
Adr. wt	Decr**	N	Incr R	Decr**	N	N
Gon. wt	Decr**R	N	Decr**	Decr**	Decr	Decr**R
Pros./Uter. wt	Decr R	Decr*	Decr	Decr	N	N
Vag. open	—	—	—	Late**	Late**	Late**
1st Est.	—	—	—	Late**	Late**	Late**
Cycles	—	—	—	Longer**	N	Longer**
Pit. TSH	Decr**	N	N	Decr*	N	N
Serum TSH	Decr**	Decr**	Decr	Decr**	Decr	Decr

[a] Only the F_1 and F_2 offspring of neo-T4 mothers are shown. The F_2 are the offspring of the female F_1 shown here. Significances are as presented in Table 7.

TABLE 12. *Untreated Male F_1 and F_2 progeny of neo-T4 mothers*

	F_1 Co	F_1 T4	F_2 Co	F_2 T4
Age (days)	114	114	108	108
Final N	10	10	10	10
Eye-opening (days)	14.9 ± 0.1	14.2 ± 0.2**	14.6 ± 0.3	14.5 ± 0.1
Wean wt (g)	51 ± 2	48 ± 1	50 ± 2	52 ± 1
Final wt (g)	474 ± 9	435 ± 28	497 ± 11	448 ± 9**
Pit. wt (mg)	11.72 ± 0.24	11.33 ± 0.51	11.69 ± 0.45	11.98 ± 0.45R
Thy. wt (mg)	14.3 ± 0.9	16.9 ± 1.0R	15.0 ± 0.7	17.3 ± 0.8*RR
Adr. wt (mg)	46.0 ± 1.8	48.3 ± 3.9	44.1 ± 1.9	45.9 ± 1.6R
Gon. wt (g)	3.46 ± 0.08	3.45 ± 0.17	3.94 ± 0.05	3.67 ± 0.06**
Pros. (mg)	623 ± 16	540 ± 36*	579 ± 35	521 ± 15
Pit. TSH (mU)	266	239	349 ± 28	332 ± 41
SME-TSH (μU)	186 ± 51	177 ± 39	—	—
Serum TSH (μU/ml)	62.5	50.8	72.1 ± 8.3	53.3 ± 12.6
PTU response[a]:				
Pit. TSH (mU)	40	37	48 ± 6	52 ± 6
Serum TSH (μU/ml)	279	255	165 ± 17	198 ± 29
Thy. wt (mg)	33.8 ± 1.7	35.6 ± 1.5	30.3 ± 0.9	32.7 ± 1.1

[a] PTU, 0.00625% drinking water for 7 days.

The F_2 are the offspring of F_1 mothers and control fathers. Note the close similarity of the F_1 and F_2 controls.

Significances are as presented in Table 7.

VII. STUDIES OF UNTREATED F_1 OFFSPRING OF THYROIDECTOMIZED MOTHERS

Methods

Abnormalities in the untreated offspring of neo-T4 and neo-PTU mother rats raised the question of whether or not these changes might be caused by the hypothyroid state of the mother. To study this possibility, rats 55 ± 2 days of age were placed on a low-iodine diet for 4 days and then injected subcutaneously with 500 μCi of ^{131}I. Twenty-eight days after radiothyroidectomy they were caged with normal rats of opposite sex for 16 days. Five to six females were caged with two males. The males were rotated every few days to minimize the effect of an infertile male.

Results

Table 13 shows that mating of controls with thyroidectomized males produced a 100% pregnancy rate without any maternal mortality and the pup mortality at birth was negligible (one death in 177 pups). By contrast the thyroidectomized females had a pregnancy rate of only 87% and seven of the 26 mothers (27%) died during labor. In addition there was an 11% mortality of the pups (excluding the pups of the seven mothers that died during labor). The thyroidectomized mothers also had smaller litters; sub-

TABLE 13. *Fertility of thyroidectomized parents*[a]

	Co F × Co M	%	Co F × Thyx M	%	Thyx F × Co M	%
Pregnancy rate	11/11	100	14/14	100	26/30	87
Maternal mortality[b]	0/11	0	0/14	0	7/26	27
Pup mortality[c]	0/119	0	1/177	< 1	13/123	11
Average litter size[d]	10.8 ± 1.2 (2–15)	—	12.6 ± 0.9 (4–16)	—	6.5 ± 0.3 (4–9)	—
Pup mortality[e]	3/80	4	0/40	0	14/95	15

[a] Breeding exposure was 16 days. 5–6 F were caged with 2 M (M = male; F = female). To promote lactation it was necessary to give the Thyx F 1 μg T4/100 body wt every 3 days for six doses (18 days).
[b] During parturition.
[c] At birth (excluding the seven mothers that died).
[d] Mean ± SE: range in brackets.
[e] Mortality after birth until weaning. Total *N* adjusted at birth to stated *N*.

sequent pup mortality after birth until weaning was 15% in this group and negligible in the offspring of the controls and thyroidectomized fathers.

When the thyroidectomized parents were killed at 195 days of age, their body weights were significantly reduced in both sexes, and pituitary weight was significantly increased in the males but not the females. Adrenal weights were significantly reduced in both sexes; testicular and ventral prostate weights were significantly increased. The pituitary TSH content was significantly reduced in both sexes; the SME-TSH content was reduced significantly in the males and serum TSH was markedly elevated in both sexes as would be expected. After stimulation with TRH, there was a marked augmentation in the serum TSH response as is characteristic of hypothyroidism. Within 5 min of administering TRH (50 ng/100 g bw), serum TSH rose from 106 to 1,151 mU/100 ml in the thyroidectomized rats, as compared with 9.7 to 21.7 mU/100 ml in the controls.

Table 14 summarizes the studies on the untreated offspring of the hypothyroid mothers. It may be seen that the females showed a greater delay in eye-opening than males but that both sexes had a significant reduction in weaning weight as well as in final body weight. Although pituitary weights were not abnormal, absolute and relative thyroid weights were significantly increased in both sexes. Adrenal weights were decreased in the males and ovarian weights decreased in the females but uterine weight, vaginal opening, and the estrous cycles were not abnormal. There were no abnormalities in the serum T4 concentration, pituitary TSH content, and SME-TSH content, but there was a significant increase in serum TSH in the males. The TSH response to TRH stimulation was normal. PTU (0.00625% in the drinking water) was administered for 7 days, and the depletion of pituitary TSH content, the marked rise in serum TSH concentration, and the growth of the goiter were all within normal limits in the offspring of the hypothyroid mothers.

TABLE 14. *Late effects of maternal hypothyroidism on their progeny*[a]

Treatment	Co	Hypo Ma	Co	Hypo Ma
Sex	Male	Male	Female	Female
Final N	24	23	12	12
E.O. (d)	14.9 ± 0.2	15.2 ± 0.2	14.3 ± 0.1	15.3 ± 0.2**
Wean wt (g)	52.4 ± 1.1	43.1 ± 0.9**	54.4 ± 1.0	40.6 ± 1.1**
Final wt (g)	435 ± 6	390 ± 7**	260 ± 5	230 ± 6**
Pit. wt (mg)	11.2 ± 0.25	10.6 ± 0.33	14.0 ± 0.49	12.2 ± 0.62*
Thy. wt (mg)	13.7 ± 0.3	15.8 ± 0.5**RR	12.4 ± 0.5	14.2 ± 0.5*RR
p PTU	—	—	27.0 ± 0.77	29.0 ± 1.56RR
Adr. wt (mg)	44.0 ± 0.8	40.7 ± 1.1*	61.4 ± 2.1	58.4 ± 2.5
Testes wt (g)	3.61 ± 0.15	3.41 ± 0.11	—	—
Ovary wt (mg)	—	—	83.7 ± 3.4	73.9 ± 2.4*
Pros. wt (mg)	491 ± 24	517 ± 20RR	—	—
Uter. wt (mg)	—	—	462 ± 22	419 ± 25
Vag. open (days)	—	—	31.0 ± 0.3	30.9 ± 0.6
1st Est. (days)	—	—	31.3 ± 0.3	31.5 ± 0.7
Cycles min (days)	—	—	3.7 ± 0.1	3.7 ± 0.1
max (days)	—	—	4.5 ± 0.1	4.6 ± 0.1
Serum T4 (μg%)	6.16 ± 0.44	6.06 ± 0.64	4.24 ± 0.45	4.60 ± 0.56
Pit. TSH (mU)	292 ± 47	327 ± 32	234 ± 21	238 ± 20
p PTU	—	—	51 ± 5	45 ± 7
SME-TSH (μU)	220 ± 32	194 ± 48	126 ± 55	78 ± 26
p PTU	—	—	73 ± 21	116 ± 37
Serum TSH (μU/ml)	54 ± 6	84 ± 7**	52 ± 18	39 ± 8
p PTU	—	—	187 ± 20	213 ± 33
8′ *p* 50 ng TRH/100 g	758 ± 50	810 ± 69	—	—

[a] Mothers radiothyroidectomized 28 days prior to mating. To assist lactation they received T4, 1 μg/100 g bw the 1st day post partum and 0.5 μg/100 g bw the 2nd day, and none thereafter. Offspring not treated. Males killed when 94 days old; females at 100 days.

Discussion and Conclusion

The reduced body weight both at weaning and throughout adult life is probably attributable to intrauterine and postpartum malnutrition. The same abnormalities are seen when normal neonatal rats are subjected to underfeeding by manipulating litter size (see Sec. III). These same changes are also noted in neo-T4 and neo-PTU rats, although the mechanism is probably not due to neonatal malnutrition in these cases. A delay in eye-opening is noted in both neo-PTU rats and neounderfed rats but it is accelerated in neo-T4 animals. There is no effect on the timing of puberty or on estrous cycles in the offspring of the thyroidectomized mothers. These observations contrast with the delayed puberty and prolonged cycle length seen in both neo-T4 and neo-PTU rats. The offspring of the thyroidectomized mothers differed significantly from those who suffer neonatal underfeeding. The underfed animals had normal thyroid weights, whereas the offspring of thyroidectomized rats had significantly increased thyroid

weights both absolutely and relatively. In the case of the males, this was associated with a significant increase in serum TSH concentration, even though the serum T4, pituitary TSH, and response to challenge with TRH or PTU were all normal.

It is concluded that even though perinatal malnutrition was present in the offspring of hypothyroid mothers, the offspring showed changes that cannot be ascribed to malnutrition. The most interesting of these changes include the enlargement of the thyroid glands. The milk from the thyroidectomized mothers may have been deficient in certain organic iodine compounds but certainly not in inorganic iodine, since they were eating rat chow with a high iodine content. The normal pituitary TSH content, serum T4 concentration, and normal response to TRH all preclude primary hypothyroidism being present in the offspring. The elevated serum TSH in the male offspring is unexplained. Possibly in this group there was a mild thyroidal defect reducing thyroid reserve and associated with a compensatory increase in TSH secretion. How hypothyroidism in the mother could cause a thyroidal defect in her offspring is not at all clear. Possibly transplacental transfer of maternal TRH is involved.

VIII. THE CROSS-FOSTERING OF UNTREATED F_1 OFFSPRING OF NORMAL AND THYROIDECTOMIZED MOTHERS

Using the same type of protocol as described in Table 9, radiothyroidectomized mothers were used in the experiment shown in Table 15. The only difference between this and the experiment shown in Table 14 is that the nursing thyroidectomized mothers received 1 μg of T4/100 g bw subcutaneously every 3rd day to prevent inadequate lactation. This dose was selected to ameliorate, but not correct, the maternal hypothyroidism.

Results and Discussion

Table 15 shows that it is the thyroid status of the lactating mother that determines weaning weight. In the males the weaning weight was reduced regardless of whether the pups originated from a thyroidectomized mother or a control mother as long as the nursing was by a thyroidectomized mother. This was the only parameter so clearly related to the status of the nursing mother. It may be seen that the male offspring of thyroidectomized mothers had smaller pituitary glands whether they were nursed by thyroidectomized mothers or control mothers. In both sexes thyroid weights were larger in the offspring of thyroidectomized mothers whether reared by a control or by a thyroidectomized mother. The difference was only significant when the F_1 of thyroidectomized mothers were nursed by control mothers. Possibly abundant nutrition is essential to show this effect. The females confirm this interpretation and show that the pituitary weight re-

TABLE 15. *Cross-fostering untreated (F_1) offspring of normal and thyroidectomized mothers*

	Co Co[a]	Tx Tx[b]	Co Tx	Tx Co	Co Co	Tx Tx	Co Tx	Tx Co
Sex	Male	Male	Male	Male	Female	Female	Female	Female
Final N	14	17	14	15	15	14	14	10
Eye-Opening (d)	15.5	15.0	15.2	14.9	15.1	14.6	14.7	15.0
Wean wt (g)	44.2	32.7**	32.3**	49.2	44.6	31.2**	28.2**	44.7
Final wt (g)	431	432	414	448	261	251	253	272
Pit. wt (mg)	11.95	10.41**RR	11.47	10.95*RR	14.68	12.79*R	14.02	12.53*RR
Thy. wt (mg)	16.7	18.0	17.4	19.8**RR	13.1	15.1*RR	13.4	14.8*
Adr. wt (mg)	45.9	47.1	38.6*RR	43.3R	61.8	66.5	57.5	72.7**R
Gon. wt (g, mg)	3.90	3.16**RR	3.37**	3.61*RR	80.0	76.9	79.6	79.8
Pros./Uter. wt (mg)	485	575	502	537	540	514	482	530
Pit. TSH (mU)	255	279	273	278	259	273	215	223
Serum TSH (μU/ml)	40.4	58.8*	66.0*	61.8*	41.5	40.1	36.9	47.4
15′ $\bar{p}$ TRH (μU/ml)	770	728	727	766	461	431	436	553

[a] Co Co indicates control F_1 with control mother.
[b] Tx Tx indicates F_1 pup from thyroidectomized mother, fostered by a thyroidectomized mother.
All rats were killed at 124 days of age.

sponse was the result of prenatal influences and not affected by postnatal care. The serum TSH was elevated in the male offspring of thyroidectomized mothers as well as in the group of male controls nursed by thyroidectomized mothers, confirming the results shown in Table 14. Thus the thyroid status of the nursing mother was responsible for a permanently elevated serum TSH in the offspring. The response to TRH was normal in all groups.

Thus as suggested by the results observed in studies of the cross-fostering of offspring of neo-T4 mothers, these results indicate that some of the abnormalities in the pups are not the result of abnormal maternal nursing care in the neonatal period but depend upon some influence prior to birth.

IX. STUDIES OF THE UNTREATED F_1 OFFSPRING OF NEO-T4 FATHERS

Background

It was our working hypothesis that the abnormalities in the untreated offspring of neo-T4 mothers, neo-PTU mothers, and thyroidectomized mothers were the consequence of intrauterine and nursing abnormalities reflecting the metabolic status of the mother. Searching for indirect evidence for this hypothesis, we studied the offspring of neo-T4 males. Of course these fathers had no contact with their offspring. To our great surprise we found, and later confirmed, the presence of abnormalities in the offspring of neo-T4 fathers. Table 16 shows one of these experiments comparing the untreated F_1 of simultaneously studied control groups and the untreated F_1 of neo-T4 fathers mated with normal mothers.

Results

There were no differences in age of eye-opening, weaning weight, vaginal opening, or first estrus. Ovarian weights were significantly larger in the F_1 of neo-T4 fathers when corrected for body weight. Pituitary and thyroid weights were significantly larger both absolutely and relatively in male offspring of neo-T4 fathers. The thyroids of the female offspring of neo-T4 fathers were also larger but the pituitaries were not. Pituitary TSH content and serum TSH levels were higher in both male and female offspring of neo-T4 fathers as compared to offspring of controls, but the differences were not statistically significant. The TRH response 15 min after the i.v. injection of 100 ng/100 g bw was significantly blunted in male offspring of neo-T4 fathers.

Discussion

It is of possible significance that some of the changes in the untreated progeny tend to be the opposite of those observed in the neo-T4 fathers

TABLE 16. *The untreated F_1 of controls versus untreated F_1 of Neo-T4 fathers and normal mothers*[a]

	F_1 Males		F_1 Females	
	Control	Neo-T4 Father	Control	Neo-T4 Father
Final N	8	10	7	10
Eye-opening (days)	15.0	15.2	14.6	14.9
Wean wt (g)	46.4	46.1	43.1	43.5
Final wt (g)	468	479	248	266*
Vag. open (days)	—	—	35.3	35.9
1st Estrus (days)	—	—	35.7	36.0
Pit. wt (mg)	11.4	12.8**R	15.2	13.6RR
Thy. wt (mg)	15.2	19.9**R	13.0	16.1*R
Adr. wt (mg)	45.0	52.4*	66.9	58.8R
Gon. wt (g/mg)	3.69	3.89	81.8	89.5R
Pros. wt (mg)	572	478	—	—
Uter. wt (mg)	—	—	538	593
Pit. TSH (mU)	246	312	223	244
Serum TSH (μU/ml)	50.1	82.0	41.7	50.8
15′ $\bar{p}$ TRH (μU/ml)	1111	721**	587	593
PTU Response				
Pit. TSH (mU)	59	77	58	60
Serum TSH (μU/ml)	304	455	452	358
Thy. wt (mg)	33.8	43.9*	43.2	37.8

[a] These rats were killed when 134 days old.

themselves. The fathers had smaller pituitaries, thyroids, adrenals, and gonads; their pituitary and serum TSH concentrations were reduced and their response to PTU challenge showed a blunting of the depletion of pituitary TSH, the rise in serum TSH, and growth of the goiter. By contrast, the untreated offspring had increased pituitary and serum TSH concentrations, and the response to PTU challenge showed an augmentation of serum TSH concentrations and thyroid weight gain as compared with the controls. Only in their blunted response to TRH stimulation did the offspring share a response similar to that seen in their fathers.

On the other hand, there are certain similarities between these untreated offspring and neo-PTU rats (see Table 7). Both showed increased thyroid weights, pituitary and serum TSH concentrations were increased, and the TSH response to PTU challenge in adulthood was augmented. The blunted response to TRH seen in neo-PTU animals also was similar to that seen in the offspring of neo-T4 fathers. There were many more similarities with neo-PTU rats than with the offspring of neo-T4 mothers (see Table 18).

It would appear that the untreated F_1 of neo-T4 fathers had an augmented secretion of TSH with a resulting increase in pituitary and thyroid weight and an augmented response to PTU challenge. These results were not the result of primary thyroid hypofunction because the response to TRH was blunted rather than augmented. It is theorized that the thyrostat set point in these animals had been altered.

X. STUDIES OF THE UNTREATED F_1 PROGENY OF THYROIDECTOMIZED FATHERS

Using the methods detailed above (Sec. VII), male rats were thyroidectomized and 2 months later were caged with normal control females, 130 days of age and one pair per cage, for 2 weeks. There was never any contact between the males and their offspring.

Results

Table 17 shows the results when these offspring were allowed to mature and were killed at about 125 days of age. Male offspring showed a significant delay in eye-opening and an increase in final body weight. The females showed a significant diminution in weaning weight. Pituitary weight was increased in both sexes, $p < 0.05$ in males. The thyroid weights were increased in both sexes, absolutely and relatively in the females ($p < 0.01$). The testes were smaller and the ovaries larger. Serum TSH was decreased in the offspring of the hypothyroid fathers but the differences were not significant statistically. The response to TRH tended to be blunted in the male offspring of the thyroidectomized fathers and was normal in the females.

Discussion

One may speculate about the possible significance of these changes. The enlarged thyroid glands with diminished circulating TSH and normal response to TRH stimulation would seem to exclude primary hypothyroidism. Since all groups had normal lactating mothers, one cannot explain the

TABLE 17. *Untreated F_1 of controls versus untreated F_1 of thyrex fathers and normal mothers*[a]

	F_1 Males		F_1 Females	
	Control	Thyrex Father	Control	Thyrex Father
Final N	14	15	15	15
Eye-opening (days)	15.5	16.1*	15.1	15.3
Wean wt (g)	44.2	44.7	44.6	39.1**
Final wt (g)	431	471**	261	265
Pit. wt (mg)	11.95	12.84*	14.68	15.41
Thy. wt (mg)	16.7	18.0	13.1	15.2**RR
Adr. wt (mg)	45.9	46.6	61.8	61.7
Gon. wt (g/mg)	3.90	3.82R	80.0	88.5*
Pros./uter. wt (mg)	485	536	540	458*RR
Pit. TSH	255	281	259	267
Serum TSH (μU/ml)	40.4	35.3	41.5	29.5
15′ $\bar{p}$ TRH (μU/ml)	770	599	461	489

[a] The hypothyroid father never had contact with the F_1 offspring. The rats were killed when approximately 125 days old.

TABLE 18. *Comparison of the untreated F_1 progeny of parents treated neonatally*[a]

	Neo-T4 Mothers	Thyrex Mothers	Neo-PTU Mothers	Neo-T4 Fathers	Thyrex Fathers
Eye-opening	Early**	Late* or N	N	Late or N	Late* (M)
Wean wt	Decr** or N	Decr***	Decr** (M)	N	Decr** (F)
Final wt	Decr	Decr* or N	N	Incr* (F)	Incr** (M)
Vag. open	Late**	N	Late*	N	—
Est. cycles	Longer**	N	N	—	—
Pit. wt	N	Decr**	Decr*R(F)	Decr**R	Incr* (M)
Thy. wt	IncrR	Incr**RR	Decr*	Incr**R	Incr**RR (F)
Adr. wt	N	Decr** or N	Decr**R (M)	Incr (M)	N
Gon. wt	N	Decr**RR (M)	Decr**R	N IncrR (F)	DecrR (M) Incr* (F)
Pros. wt	Decr*	Incr**	Decr*	Decr	Incr
Uter. wt	N	N	Incr	N	Decr*RR
Pit. TSH	N	N	Incr	Incr (M)	N
SME-TSH	Decr	Decr	Decr*	—	—
Serum TSH	Decr**	Incr** (M)	Incr	Incr	Decr
Serum T4	—	N	—	—	—
TSH half-life	—	N	—	—	—
TRH response	Decr*	N	N	Decr* (M)	Decr (M)

[a] See footnotes to Table 7.

differences in eye-opening and body weight on inadequate maternal care. Table 18 shows that there are certain similarities and differences between the progeny of thyroidectomized fathers and the progeny of neo-T4 fathers. It should be noted that the thyroidectomized fathers were much more severely hypothyroid than the neo-T4 fathers. The radiation damage to parathyroid function and possibly other tissues may also have to be evaluated in the thyroidectomized rats. Regardless of what these changes may represent, the most surprising thing is that there are any changes at all. It is difficult to imagine a mechanism that would explain such changes independent of the influences resulting from an abnormal intrauterine environment, or poor neonatal care, or both. Spergel, Levy, and Goldner (1971) studied the progeny of rats treated as weanlings with a single subdiabetogenic dose of alloxan. This treatment was not sufficient to produce diabetes in these rats, yet their offspring manifested abnormal glucose tolerance. This defect was transmitted over several generations and appeared to increase in severity with each generation. The fact that the male parent, treated as a weanling, was as capable of transmitting the defect as the female ruled out the possibility that the fetus was affected by an abnormal intrauterine environment or by a diabetogenic milk factor. The authors postulated that the transmission occurred by a process of "paramutation" (Brink, Styles, and Axtell, 1968), which involves an acquired constraint on gene expression without altering the structure of the genome. Goldner suggested that alloxan treatment permanently altered the function of a regulator

gene(s) and that this alteration could be transmitted to future generations. These investigators have continued their experiments and confirmed the original observations through the F_6 generation (*personal communication*, 1974).

In an unrelated study, Friedler and Cochin (1968) reported that the treatment of female rats with morphine prior to their being mated affected the response of their offspring to subsequent challenge with morphine. They found some accommodation to an initial test dose of morphine in the offspring, although they had never had prior exposure to the drug. The offspring were also significantly smaller. The authors suggest that morphine may have "induced an effect on the hypothalamic-hypophyseal axis."[1]

The report by Goldsmith, McAdams, Larsen, MacKenzie, and Hess (1973) of a family manifesting three generations with various thyroid disorders may be pertinent. They concur with the suggestion of Blizzard et al. (1960) that it is necessary to postulate both a genetic element and the transplacental transfer of an unidentified thyrocytotoxic factor (possibly in addition to thyroid antibodies) to account for familial clustering of cretinism. They conclude there is presumably a genetic element, but the role of inheritance in thyroid disorders remains undefined, in part because of the difficulty in distinguishing genetic from nongenetic congenital dysfunction. Our experimental observations on the untreated progeny of hypothyroid fathers adds new data and raises new questions in this puzzling area.

XI. SUMMARY AND CONCLUDING REMARKS

It is obvious from the work of others (Barraclough, 1961; Harris, 1964) that the hypothalamic set point for the regulation of gonadal function can be permanently altered by endocrine manipulations during a critical neonatal period. We have demonstrated an additional variety of permanent endocrine regulatory abnormalities resulting from various endocrine and nutritional manipulations during the perinatal period. In the case of the neo-T4 syndrome, we have by several independent means shown that the neonatal treatment produced its effects by specifically altering hypothalamic function, apparently leading to impaired TRH secretion.

It may be speculated that hypothalamic alterations may have been induced in the other neonatal syndromes presented, although no direct evidence is available. By experiments utilizing cross-fostering, it was shown that some of the effects are produced by prenatal influences and other effects

[1] Since preparing this manuscript, Friedler has reported that the F_2 progeny show similar abnormalities and the untreated progeny of males treated with morphine prior to mating normal females also show growth failure! They were as surprised and dismayed over their results as we are over ours (In: *Perinatal Pharmacology: Problems and Priorities*, edited by J. Dances and J. E. Hwang. Raven Press, New York, 1974).

by neonatal influences, presumably inadequacies in nursing. The possibility of analogous disorders in humans must be considered.

In addition to these disorders resulting from neonatal treatment, we have also observed abnormalities in their untreated offspring, F_1, and in the following generation, F_2. This, combined with the finding of abnormalities in the untreated progeny of neo-T4 and hypothyroid fathers, raises serious and perplexing questions for those studying the causes of congenital disorders.

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DISCUSSION

Carlson: You showed some effects of both over and underfeeding in your studies. Have you done any experiments to study transmission of these abnormalities?

Bakke: We have not, but I suspect that these defects also might be transmitted.

Brasel: Zamenhof, van Marthens, and Gravel (*Science,* 172:850, 1971) have shown that mothers who are malnourished during their early life give birth to pups who are smaller in the F_1 and F_2 generations. There was no transmission by the father; the abnormalities were seen only on the maternal side.

When the paper of Shambaugh and Wilber (*Endocrinology,* 94:1145, 1974) was published, we were interested in their finding of low T4 levels in the undernourished offspring. We had produced malnutrition by increasing litter size and decreasing maternal protein intake, which produced severe growth retardation. T4 by column at the time of sacrifice was no different from control levels in these animals. Shambaugh and Wilber (1974) produced malnutrition by removing the pups from the mother for a certain number of hours each day; there might be differences in the type of malnutrition produced in this way. I would worry that reduced body temperature or cold stress, which could occur when pups are separated from their mothers, might have influenced thyroid function. This is not a factor when malnutrition is produced by increasing litter size and reducing protein intake.

Bakke: I agree with that, but remember they killed their pups at 16 days of age; things might be different at that point.

Brasel: We killed the rats weekly from 0 to 35 days of age, and each time blood was pooled for T4; no differences were found between the malnourished and control animals.

Bakke: These data show that some endocrine organs like the testes are actually larger. Also on this table we give our T4 values and none was significantly altered.

Brasel: Certainly with early postnatal malnutrition, although brain weight and DNA are reduced, relative weights are not as severely affected as body weights.

Chopra: How are you currently measuring stalk median eminence TSH? Are you using a bioassay or do you have a specific radioimmunoassay?

Bakke: We use an immunoassay procedure.

Chopra: Is this the same immunoassay that you use for pituitary TSH?

Bakke: Yes, and for serum TSH.

Chopra: Your neo-T4-treated rats had decreased pituitary TSH but increased stalk median eminence TSH. In spite of this, their thyroid function was decreased compared to the control animals. Do you believe the stalk median eminence TSH has biologic significance?

Bakke: The total stalk TSH content is small; it probably doesn't contribute significantly to changes in pituitary TSH, but you may recall that, in our first report on this stalk TSH, we noted several circumstances (hypothyroidism and T4 treatment) in which it changed in the opposite direction from the pituitary TSH content (*Neuroendocrinology,* 2:315, 1967). We have not made any further progress in trying to demonstrate whether or not this TSH has any biologic meaning.

Chopra: We studied some severe protein-calorie malnourished human adults before and after 2 months of treatment and did not find any change in total serum T4 concentration before and after treatment.

Fisher: What did the treatment include, Dr. Chopra?

Chopra: We prescribed a high-calorie high-protein diet. The free T4 was elevated when they were malnourished and decreased towards normal when they were fed.

Gardner: I would like to make a rather far-out clinical correlation with your underfed experiments and the association of larger testes and ovaries. The syndrome of leprechaunism is a poorly understood malnutritional syndrome of small infants in which there is evidence for some increased stimulation of both ovaries and testes. One wonders whether there might be a connection between this syndrome and what you have reported in rats.

Bakke: I cannot comment; it is a very interesting suggestion. Is their thyroid function normal?

Gardner: I am not sure.

Greer: You found that in one experiment there was an increased TRH content in the hypothalamic area. Did this hold true for the rest of the brain as well?

Bakke: It wasn't studied, but there are other reports suggesting that TRH is widespread in brain tissue.

Greer: Yes, the reports suggest that TRH exists in about equal concentrations in many areas of the brain; that is why I was curious.

Burrow: I think your data raise interesting points about thyroid hormone treatment in infants. We have been saying that if there is a question of hypothyroidism one should go ahead and give thyroid hormone therapy; perhaps this is not such a good thing.

Bakke: I would remind you that the doses of thyroid hormone that we have used are large and are not comparable to anything a pediatrician would give to a baby that he suspected of being a little hypothyroid. I really think it would be a disservice if we were to suggest that thyroid hormone in therapeutic doses might be harmful.

Fisher: It has been suggested that large doses of thyroid hormone early in infancy might help preserve CNS function in later life; treatment with large doses has resulted in premature synostosis of the cranial sutures.

Greer: What kind of dose was given to these infants?

Fisher: Three to four times the usual replacement doses; that is, two to four grains daily or 200 or 400 μg of T4 daily.

Bakke: Would you expect any harm from such doses even if the diagnosis was wrong?

Fisher: Yes, certainly we are aware of premature synostosis and accelerated bone maturation.

McKenzie: Dr. Bakke, is it necessary that you use very large doses?

Bakke: Yes. In addition, we have to give at least three doses during the neonatal period.

Hollingsworth: We have begun to worry a little about this question of titering the exact dose for replacement therapy in the hypothyroid infant. At present we are trying to monitor the dose with serum T4 and TSH measurements and with bone age, but of course bone age is rather slow in changing. We are trying to perfect our TSH assay to the point that we can actually show TSH suppression if we are overdosing. I don't know whether we will be successful, but I have the clinical impression that our hypothyroid infants are more often overtreated than undertreated.

Walfish: There also is the question of the kind of thyroid to use. Some recommend T3 as soon as the diagnosis is made in order to get a more rapid effect. Have you used T3 as well as T4 in your studies, Dr. Bakke?

Bakke: All of Eayrs work was done with T3 and our results with T4 are comparable.

Fisher: There is very little reason to use T3 in the treatment of hypothyroidism; Hayek, Maloof, and Crawford (*Pediat. Res.*, 7:28, 1973) recently have shown that if you treat a hypothyroid infant promptly with intravenous doses of thyroxine rather than oral doses (roughly the same dose over 10 days to 2 weeks) you can bring the TSH values to normal levels much more quickly. Whether 10 days makes any difference in eventual brain development is not known. I think that we should try to reproduce physiologic levels of T4, T3, and TSH as quickly as possible in these infants and try not to overshoot. There has been a tendency to overtreatment, as we have tried to keep serum T4 levels in the upper normal range.

Chopra: This has also been true in adults treated with 300 μg of T4 daily. With this dose the daily turnover rates of both T4 and T3 were markedly increased. Their tissues were metabolizing more than the normal amounts of thyroid hormones.

Dussault: The response of the pituitary to TRH would be one way to look at the adequacy of treatment.

Brasel: There is one group of patients that might be useful in assessing the long-term effects of very large doses of T4. This is the group of patients with postoperative thyroid carcinoma who are purposely treated with large doses of thyroid hormones for prolonged periods of time. I am not aware that any adverse effects have been reported, but I don't know if TRH responses or serum TSH levels have been examined.

Fisher: Assessing adequacy of therapy over the long-term is important, but the effects Dr. Bakke has been studying result from short-term treatment.

Bakke: That is correct.

Chopra: We have been studying several people who have been receiving 200 μg of synthyroid daily. Only one of 12 or 15 people studied have responded to TRH. In addition, most patients studied on 150 μg synthyroid daily have a subnormal serum TSH response to TRH.

Walfish: We have seen a number of cases with elevated serum TSH levels in whom the serum T3 concentration is normal and T4 is low. Such patients seem clinically to be euthyroid. These observations suggest to me that the pituitary is even more sensitive than the peripheral tissues to levels of circulating thyroid hormones, even more sensitive than the Achilles reflex. If you can normalize the serum TSH, the peripheral tissues probably will be satisfied.

Fisher: Suggesting that we try to normalize the serum TSH creates a problem. It might require a very different amount of hormone to lower the TSH to less than 10 μU/ml versus the mean of the euthyroid population. Dr. Dussault's suggestion that we utilize the TRH response may be more reasonable, but we need more experience and data to make such a decision.

Greer: I think you are making a great deal of something that doesn't matter too much. There are some older data indicating that if you measure the amount of thyroid hormone (in desiccated thyroid) required to suppress TSH secretion, the dose is exactly equivalent to $^1/_{10}$ of a milligram of T4. Roughly $^1/_3$ of the people will require one grain of thyroid a day to suppress their TSH and so on. People have been treated with three grains of thyroid a day as standard replacement for 50 years or more and have gotten along perfectly well.

Fisher: But the question of whether it is harmful to give relatively large doses of T4 to a newborn may be a different problem. First, it is much easier to give an ex-

cessive dose to an infant than an adult, and the untoward effects are very different. Accelerated growth, acceleration of bone age, premature synostosis of the cranial sutures, and perhaps some of the changes that Dr. Bakke has discussed would not be expected in adults. Some of these effects can occur with doses three or four times normal.

Greer: Do infants with neonatal thyrotoxicosis develop premature synostosis?

Fisher: They do if the thyrotoxicosis persists long enough. It requires 5 or 6 months of excessive therapy or secretion to produce premature synostosis in an infant.

Hershman: With the improvement in techniques for measuring T4 and TSH, I think you could monitor therapy quite well in infants. The sensitivity of the TSH assay has improved to the point where one could try to avoid a high T4 and a low TSH.

McKenzie: The dangers of perinatal androgen treatment have been recognized for some time. Have any of the children exposed to androgens been followed through into adulthood? Do they produce abnormal children or can they produce children?

Gardner: There are a number being followed. I am speaking of girls with adrenal hyperplasia who were exposed to their own androgens during the neonatal period. I think, in general, the impression is that these girls are different from other girls behaviorally. At least that is my impression. Probably it is related to their neonatal androgen exposure.

Burrow: Is their IQ higher?

Gardner: John Money says it is.

Brasel: Such girls certainly cycle less regularly postpubertally than normal females, but they can become pregnant.

Gardner: This is the original patient, not the F_1 generation?

Brasel: That is right.

McKenzie: This would suggest that we should be careful with the neonatal T4 dosage.

Gardner: I think so, too.

Koenig: The neonatal rat corresponds to the human fetus *in utero*. I do not think the danger of treating the newborn human would be the same as for the rat.

Bakke: The infants to study are the ones who have had neonatal thyrotoxicosis to see whether they have a normal onset of puberty and normal endocrine function. That would be the interesting group.

Koenig: According to what you have shown, I would not be too much concerned.

Brasel: These data certainly suggest that the hypothalamus can be imprinted at critical stages with regard to TRH release, just as it can with regard to gonadotropin release. How the effect is transmitted to the F_1 and F_2 generations is a very exciting question.

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Maternal Hypothyroxinemia: Development of 4- and 7-Year-Old Offspring

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In a study of thyroid function during pregnancy, begun in 1962 at Providence Lying-In Hospital, every second, fourth, or eighth prenatal registrant was included. Also patients with a history of thyroid dysfunction were enrolled in the clinical sample, not in the control group. A patient studied in one pregnancy automatically was restudied in any subsequent pregnancy. Unexpectedly hypothyroxinemia occurred in about 3% of the pregnancies monitored.

There has been much interest in the effect of maternal hypothyroidism on fetal development, particularly CNS development (Graham and Blizzard, 1973; Stanbury and Kroc, 1972), but adequate data have not been available. Therefore the offspring of these women, some treated adequately and some inadequately, have been followed carefully with regard to their CNS function. The present report summarizes studies of IQ measured in these offspring at 4 and 7 years of age.

SUBJECTS AND METHODS

Euthyroid Mothers

When these studies were begun at Providence Lying-In Hospital in 1962, radioimmunoassay techniques for measurement of serum thyroxine (T4), free thyroxine (FT4), tri-iodothyronine (T3), free tri-iodothyronine (FT3), thyroid-stimulating hormone (TSH), and thyrotropin-releasing hormone (TRH) had not been developed (Abuid, Stinson, and Larsen, 1973; Fisher, 1973; Abuid, Klein, Foley, and Larsen, 1974; Erenberg, Phelps, Lam, and Fisher, 1974). We were employing the butanol-extractable iodine (BEI) technique for measurement of serum thyroxine-like compounds (Man and Bondy, 1957). More recently we have compared BEI measurements with measurements of T4-iodine (T4) determined in Benotti's laboratory using a modification of the Murphy-Pattee displacement technique (Cassidy, Benotti, and Peno, 1968). In 13 sera the mean of calculated T4I by Benotti's

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