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Analysis of the Influence of Thyroid Hormone on Prenatal and Postnatal Maturation of the Rat [1]

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ABSTRACT The effects of hyper- and hypothyroidism on fetal development and on the postnatal maturation of the central nervous system was studied in the rat. Our observations indicated that the development of the rat fetus, as measured by birthweight and skeletal maturation of the newborn animal is not markedly influenced by presence or absence of thyroid hormone during the prenatal stage.

Availability of thyroid hormone is a more critical factor with respect to maturation of the nervous system taking place in the postnatal animal.

Delay in cerebellar maturation and delay in increase in dry weight of cerebrum and cerebellum were noted in hypothyroid rats. A transitory acceleration of oxygen consumption of these structures during their maturation was also noted in hyperthyroid litters.

A more permanent suppression of learning behavior and of the thermoregulating mechanism was observed in young hypothyroid rats deprived of thyroid hormone since birth.

It is concluded that requirements for thyroid hormone during development of the rat are limited to a critical period coinciding with the first two to three weeks of postnatal age.

The problem of the influence of thyroid hormone on processes of growth and differentiation has a considerable background in the literature. For a review see Eleftheriou ('62), Etkin ('64), Osorio and Myant ('60) and Pitt-Rivers and Tata ('59).

The role of the thyroid gland for maturation of the skeletal and the nervous systems has been particularly emphasized in the experimental literature. See Barnett ('48), Bradley, Eayrs, Glass and Heath ('61), Eayrs ('53, '54, '55, '59, '60), Eayrs and Horn ('55), Eayrs and Lishman ('55), Eayrs and Taylor ('51), Fell and Mellanby ('55, '56), Hamburgh ('55), Hamburgh and Flexner ('57), Hamburgh and Vicari ('57), Hammett ('23, '24, '26), Horn ('55), Hunter and Sawin ('42), Hughes ('44), Kollross ('42), Kollross and Peppernits ('52), Liddell ('23, '26, '27), Liddell and Simpson ('23, '26), Smith and McLean ('38), Todd, Wharton and Todd ('38), Weiss and Rossetti ('51). Little can be said with certainty concerning the mechanism by which thyroid hormone exerts its influence on development and maturation on several vertebrate structures and tissues.

In warm blooded animals where it is difficult to separate the metabolic from the developmental effects of the hormone, attempts to explain the morphogenetic changes associated with hypothyroidism have suffered from contradictory results and conclusions relating mainly to variability in timing and duration of experimental treatment, transplacental passage of thyroid hormone and reversibility and irreversibility of the effects of of thyroid deprivation during growth and development.

The present study was undertaken to obtain answers to the following specific questions:

(1) Is thyroid hormone essential in the rat for fetal development, or for postnatal development, or for both?

(2) Are the needs for thyroid hormone during maturation of the rat limited to a specific "critical period," that can be defined?

(3) Are the effects taking place in the absence of thyroid hormone during development transitory, persistent and/or irreversible in nature?

Thyroid needs during fetal development were measured by comparing body weights and skeletal maturation in newborn rats

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obtained from hypothyroid, hyperthyroid and control mothers.

Requirements for and effects of thyroid hormone during postnatal development were studied by investigating maturation of the central nervous system in hypo-, hyperthyroid and control rats during the first four weeks of postnatal life. This study was intentionally limited to an analysis of differentiation of the central nervous system structures, because it has been repeatedly asserted in the literature, that no tissue suffers more severely than the brain from lack of thyroid hormone during early life. The rat brain is particularly suitable material for studies of this kind, because at birth it is still very immature and its cells relatively undifferentiated.

MATERIALS AND METHODS

Materials

Pregnant rats of the Charles River Breeding Laboratories Caesarian Derived (CD) strain were used for all experiments. Animals were housed individually in an air-conditioned room and provided with a Rockland mouse diet (ground) and water ad libitum.

EXPERIMENTAL PROCEDURE

1. Determination of thyroid hormone requirements of the rat fetus

Animals were divided into three main groups. *Group 1* consisted of pregnant rats that were offered a goitrogenic diet containing 0.2% propylthiouracil (PTU) mixed into powdered Rockland mouse diet, starting on the fifteenth day of pregnancy. The fifteenth day was chosen for the initiation of experimental treatment because available evidence suggests that in the rat the fetal thyroid does not functionally mature before the eighteenth or nineteenth day of gestation, and that during the first two trimesters of the gestational period maternal thyroxin probably does not pass the placental barrier (Hamburgh, Sobel, Koblin and Rinestone, '62).

Group 2 consisted of pregnant rats offered the same goitrogenic diet, but given daily injections of l-thyroxin.[2] Thyroxin injections started on the fifteenth day of gestation and continued until delivery. Dosages administered ranged from 10, 25, and 100 μgm thyroxin dissolved in 1 ml of fluid. The rationale of this procedure to induce a hyperthyroid condition in the fetuses followed a method of hormone assay described by Dempsey and Astwood ('43). The goitrogen (PTU) that was fed caused the thyroid glands to enlarge in size, whereas the exogenous thyroid hormone, depending upon its level, offset the tendency of the thyroid to become enlarged, or if present in excess concentration would tend to diminish thyroid weight below control levels. The effectiveness of this method to assay circulating thyroid hormone in the rat fetus has been established previously by Hamburgh, Sobel, Koblin and Rinestone ('62).

Thyroxin was prepared by dissolving the sodium salt of l-thyroxin in physiological saline, adjusted to pH 8–8.5 by addition of 0.01 N NaOH.

Group 3 consisted of pregnant control rats maintained on an unsupplemented diet and receiving daily injections of 1 ml of alkaline saline, the composition of which was equal to that of the vehicle in which the hormone was dissolved.

At birth the weights of newborn litters were recorded. The litters were then sacrificed and their skeleta prepared for staining with alizarin red following a modified method of Dawson ('26).

2. Determination of requirements and effect of thyroid hormone during postnatal development of the rat

Animals were divided into several groups. *Group 1* consisted of hypothyroid rats. Hypothyroidism was induced in the manner described above. The goitrogenic diet was offered to pregnant rats on the fifteenth day of gestation and was continued to the lactating animal for 3–4 weeks after the birth of the litter.

Group 2 consisted of hyperthyroid rats. Hyperthyroidism was induced by daily administration of l-thyroxin starting at birth to rats maintained on PTU since the fifteenth day of gestation. Dosages were as follows: 1 μgm thyroxin to newborn

[2] Grateful acknowledgment is made to Smith, Kline and French for supplying thyroxin and triiodothyronine used in this investigation.

rats until age seven days, 2 μgm to rats aged 7–14 days, 3 μgm to rats between ages 14–21 days and 5 μgm to rats above 21 days of age. This concentration was established as the maximum nonlethal dose for young rats. The hormone was injected in 0.01 ml of fluid. The fluid volume was held minimal in order to prevent any interference with osmotic equilibrium in the young animals. In one series of experiments (testing oxygen consumption of brain) hyperthyroidism was induced by administration of excess triiodothyronine [3] in the same dosages to animals not previously "thyroidectomized."

Group 3 were control animals. They were maintained on an unsupplemented diet. Newborn rats were given daily injections of 0.01 ml fluid. The composition of the fluid was identical with that of the vehicle in which thyroxin was dissolved.

The differentiation of the nervous system was studied using morphological, biochemical, physiological and behavioral criteria.

For the histological analysis animals of the control and hypothyroid groups were sacrificed at five day intervals starting on the fifth day until the thirtieth day of postnatal age. Brains were removed, fixed in 10% formalin, embedded in paraffin, cut in 15 μ sagittal or transverse plane and stained with cresyl violet.

Changes of oxygen consumption of cerebral cortex, cerebellum and medulla oblongata in normal and hyperthyroid young rats were followed during the first three weeks of postnatal life. At five day intervals to coincide with 5, 10, 15 and 20 days of postnatal age the animals were sacrificed, and used for determination of O_2 consumption according to standard manometric techniques. The animals were killed by decapitation. The cerebral cortex, cerebellum and medulla were quickly dissected and 100 mg of tissue was weighed wet on a Roller Smith balance. In order to expedite the start of the experiment the tissue slices prepared with a scalpel were placed in a pre-chilled reaction Warburg vessel containing Krebs Phosphate Ringer, 2% of glucose and KCl. The total volume was adjusted to 2 ml.

Changes in dry weight/wet weight ratio of cerebellum and cerebral cortex of control and hypothyroid young rats were followed during the same period with the use of a vacuum oven set at 64°C.

The emergence of the capacity to learn a simple conditioned response was tested in control and hypothyroid young rats using the water escape response test described by Essman and Jarvik ('61).

A water-filled tank was used to test for the acquisition and retention of a simple escape response. A ramp was positioned laterally on a side opposite to the point of entry into the tank and this provided the only means of escape for the test animals. Rats were placed individually in the tank filled with water at 20°C, and given four trials to acquire the escape response. Trials were spaced 5 minutes apart, and following each, the subject was dried and returned to its home cage under a 60-w heating lamp. Response latencies were recorded for each trial; those rats showing a successive decrement in response latency with a final latency less than ten seconds were considered to have learned.

The emergence of the thermoregulating mechanism in control and hyopthyroid young rats was followed from birth until maturity. At five day intervals starting on the fifth day of age, rats were removed from their mothers and kept isolated in a small cage in a temperature controlled room set at 70°F for one hour. At the end of this time, their body temperature was recorded rectally with an electronic telethermometer.

RESULTS

1. Requirements of thyroid hormone during fetal development

The efficacy of our treatment to induce a hyperthyroid and hypothyroid state respectively in the fat fetus was tested by following changes in fetal thyroid weight, when PTU or excess thyroxin was given to the mother. Ingestion of PTU by the mother resulted in enlargement of the thyroid glands of all fetuses (fig. 1). Twenty-five micrograms of thyroxin given to the pregnant mothers maintained on a goitrogenic diet reduced the thyroid weights of newborn animals to one-half of that of untreated (control) mothers

[3] See footnote 2.

while with a dose of 10 μgm the fetal thyroids were more nearly like those of control fetuses.

Alteration of the thyroid state during the latter part of gestation of the rat had a very slight though satistically significant effect on the body weight of the newborn rat. Both removal as well as excess administration of hormone to the rat fetus lead to a slight depression of normal mean body weight in the newborn animal (see table 1). Suppression of thyroid hormone during gestation was ineffective in exerting developmental effects on skeletal maturation.

This picture of skeletal maturity is essentially the same at birth for hypothyroid litters obtained from mothers treated with PTU during the latter part of gestation, as that of control litters obtained from untreated mothers (see table 2 and fig. 5).

2. *Requirements of thyroid hormone during postnatal development of the central nervous system*

(a) *Histological analysis of brain maturation.* Structures affected in hypothyroid animals included the cerebral cortex, where our analysis confirmed the findings of Earyrs ('53, '54, '55) that there is a considerable denser packing of cell bodies per unit area of cerebral cortex. Superficial examination of the brainstem histology did not reveal any influence on the neurohistology of these structures through lack of thyroid hormone during postnatal development. The histological analysis revealed the most dramatic deviation from normal in the neurohistology of the maturing cerebellum of hypothyroid young rats (figs. 6–7). The development of the rat cerebellum during the first three weeks of age is characterized by extensive migrations of cells derived from the transitory external zone past the molecular area to form the granular layer of the cerebellum. In the rat the thickness of this external layer attains its maximum at about eight days after birth and subsequently diminishes progressively. It disappears at the twenty-first day, at which time the surface of the cerebellum has acquired the characteristic aspect of the adult (fig. 6).

Among the hypothyroid rats the superficial layer of the cerebellar cortex is maintained in the "fetal" condition as late as the twenty-sixth day of age. After that date the cerebellar cortex of hypothyroid rats begins to assume the normal architec-

TABLE 1

Body weight in grams at birth

Series	Group A Control rats	B Hypothyroid rats	C Hyperthyroid * rats	D Hypothyroid * rats given replacement thyroxin therapy
I	5.6 ± 0.14 N = 25	5.2 ± 0.17 N = 17	5.4 ± 0.05 * N = 31	
II	6.1 ± 0.06 N = 55	5.7 ± 0.10 N = 60	5.0 ± 0.18 * N = 24	5.6 ± 0.11 * N = 30
III	5.6 ± 0.07 N = 66	5.3 ± 0.06 N = 163		

* IC Normal mothers given 100 μg thyroxin.
IIC Hypothyroid mothers given 25 μg thyroxin.
IID Hypothyroid mothers given 10 μg thyroxin.

"t" tests for significance between two means

Groups	Significant difference	N	P
IA–IB	No	40	> 0.05
IA–IC	No	54	> 0.10
IIA–IIB	Yes	113	< 0.001
IIA–IIC	Yes	77	< 0.001
IIA–IID	Yes	83	< 0.001
IIIA–IIIB	Yes	227	< 0.001

TABLE 2

Osseous development in newborn rats

Group	Upper limb							Lower limb							Avg no. of caudal vertebrae
	Humerus	Radius	Ulna	Meta-carpals II–V	Carpals	Phalanges of digits II–V		Femur	Tibia	Fibula	Meta-tarsals II–V	Tarsals % of animals ∁ one tarsal bone	Phalanges of digits II–V		
						Prox	Mid Dist						Prox	Mid Dist	
Control															
N = 53	+	+	+	+	–	+	– +	+	+	+	+	28 ± 0.6	+	– +	6.3 ± 0.18
Hypothyroid															
N = 52	+	+	+	+	–	+	– +	+	+	+	+	37 ± 0.6	+ *	– +	6.3 ± 0.24

The presence of calcified bone as indicated by the Alizarin Red Stain is denoted by (+), its absence by (–). The average number of caudal vertebrae ± S.E. is also noted.

* The average number of proximal phalanges of the hypothyroid rats was 3.4 ± 0.9 out a possible four.

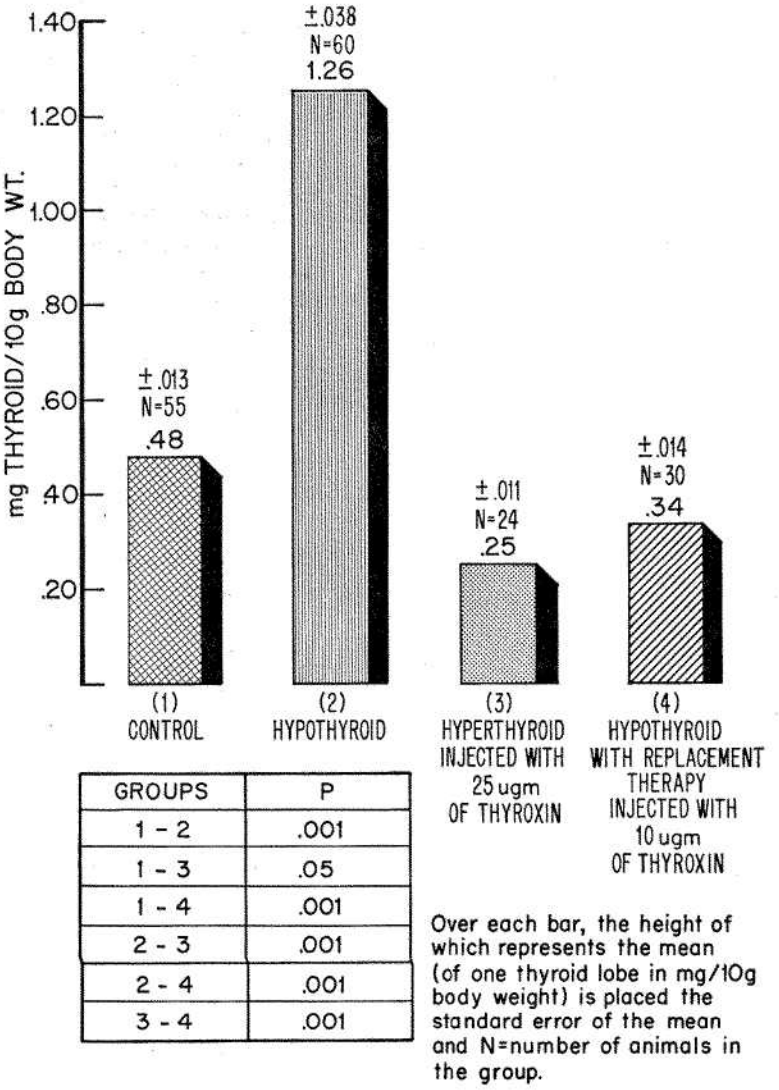
tonics, even though thyroid hormone is not made available (fig. 7).

(b) *Oxygen consumption of cerebral cortex, cerebellum and medulla oblongata.* The changes in oxygen consumption of maturing cerebral cortex and cerebellum follows a straight line until adult levels are reached between the twentieth and twenty-fifth day of life. The change in oxygen consumption of the medulla oblongata follows a straight line until the tenth day of age, when the curve levels off (fig. 2).

Administration of excess triiodothyronine started at birth and continued throughout the period of weaning increased the respiratory rate of the three brain areas tested in a significant manner only during the first week of postnatal age. Thereafter the differences in oxygen consumption of cerebral cortex, cerebellum and medulla oblongata among normal control rats and hyperthyroid rats were statistically no longer significant (see table 3).

(c) *Changes in dry weight/wet weight ratio.* Changes in dry weight/wet weight ratio are recorded in table 4. There is a consistent difference in increment of dry weight of cerebral cortex and cerebellum between 15 day and older control and hypothyroid young rats. The hypothyroid rats seem to lag behind the controls so that the 15 day old hypothyroid rat attains the level attained by the control animal at ten days of age. At 30 days the hypothyroid animal has reached the level attained by the control at 15 days. The magnitude of the difference decreases or vitrtually disappears at 40 days.

(d) *The emergence of the capacity to learn a simple conditioned response.* Figure 3 shows graphically the mean response latency shown by normal, hyper- and hypothyroid young rats at age 20 to 28 days, to learn a simple escape response from a water fillted tank during four trials. Control and hyperthyroid rats did not differ in their capacity to acquire this response. The hypothyroid animals however, failed to learn the escape response at all. A group of older hypothyroid rats maintained on the PTU diet up to 50 days of age, (not shown in fig. 3) failed to perform on this test just as completely as the younger age groups did.



GROUPS	P
1 - 2	.001
1 - 3	.05
1 - 4	.001
2 - 3	.001
2 - 4	.001
3 - 4	.001

Fig. 1 Thyroid weights of newborn control, hypothyroid, hyperthyroid and hypothyroid (with replacement therapy) rats.

(e) *The emergence of the thermoregulating mechanism.* As shown in figure 4 the ability to maintain mammalian temperatures in a cold environment is not attained in normal rats until about the thirtieth day of age.

In hypothyroid rats the mechanism responsible for thermoregulation fails to mature sufficiently to enable the animals to maintain an elevated internal temperature as long as they are deprived of thyroid hormone.

DISCUSSION

The results of our study indicate that in the rat, deprivation of thyroid hormone or excess administration of it during the intrauterine stage of development does not seem to interfere appreciably with the normal course of growth and

TABLE 3

Oxygen consumption of cerebral cortex, cerebellum and medulla oblongata of normal and hyperthyroid young rats

	5–7 Days		10–12 Days		15–17 Days		20–22 Days	
	C	T	C	T	C	T	C	T
Cerebral cortex	0.75 ± 0.03 (7)	0.86 ± 0.03 (8)	1.00 ± 0.06 (10)	1.13 ± 0.07 (11)	1.27 ± 0.07 (11)	1.41 ± 0.08 (10)	1.52 ± 0.02 (7)	1.68 ± 0.04 (7)
	[P = < 0.025]		[P = > 0.10]		[P = > 0.10]		[P = < 0.01]	
Cerebellum	0.79 ± 0.02 (9)	0.97 ± 0.02 (9)	1.06 ± 0.06 (8)	1.19 ± 0.04 (9)	1.36 ± 0.07 (10)	1.46 ± 0.08 (10)	1.50 ± 0.07 (5)	1.35 ± 0.14 (6)
	[P = < 0.001]		[P = > 0.05]		[P = > 0.30]		[P = > 0.30]	
Medulla oblongata	0.93 ± 0.02 (8)	1.03 ± 0.04 (6)	1.20 ± 0.07 (9)	1.27 ± 0.07 (8)	1.26 ± 0.06 (8)	1.36 ± 0.03 (8)		
	[P = < 0.05]		[P = > 0.40]		[P = > 0.10]			

C = control. T = Hyperthyroid. The unit recorded is μl of O_2 consumed per half hour per mg of tissue wet weight. The mean and its standard error are given; in parenthesis is the number of animals in each group. P = the probability of obtaining by random sampling the difference between means of control and hyperthyroid groups ("t" test).

maturation of the fetus up to birth, at least by the criteria used in our experimental setup, i.e. body weight and skeletal maturation. These results are in agreement with findings by Chu ('44), Davenport and Swingle ('27), Hodges, Hamilton and Keettel ('52), Moore ('50), Parkin and Greene ('43), but they are at variance with the findings of Krohn and White ('50) who showed a significantly higher rise in the occurrence of abortion and resorption if the mother was thyroidectomized. This, however, may only mean that the mother requires thyroid hormone to maintain a proper environment for the fetus. It does not prove that the fetus itself requires a supply of hormone. The only major challenge to this view is provided by the work of Weiss and Noback ('49) who have shown that if the pregnant rat is treated with thiouracil, ossification in the 16 day old rat fetus is delayed. Our own study would indicate, however, that if there is such a delay, the hypothyroid fetus of the thiouracil treated mother has caught up with the untreated control at the time of birth.

A different situation prevails if thyroid hormone is added or withdrawn after birth. Our results confirm the often stated observation, that deprivation of thyroid hormone delays and retards, but rarely suppresses the postnatal maturation of several structures, while conversely excess hormone administration may accelerate the processes of maturation.

The histogenesis of the cerebellar cortex was delayed in hypothyroid rats but the normal organization of the cerebellar cortex eventually emerged in spite of continued total deprivation of thyroid hormone. A similar observation was reported by Legrand, Kriegel and Jost ('61), and Legrand ('63).

Our demonstration of a small but statistically significant increase of oxygen consumption in brains of young (5–7 day old) hyperthyroid rats confirms results reported by Reiss, Reiss and Wyatt ('56). Failure to stimulate oxygen consumption of older hyperthyroid rats is not unexpected in view of the well estabilshed observation that the metabolism of the brain is better protected against interference than any other

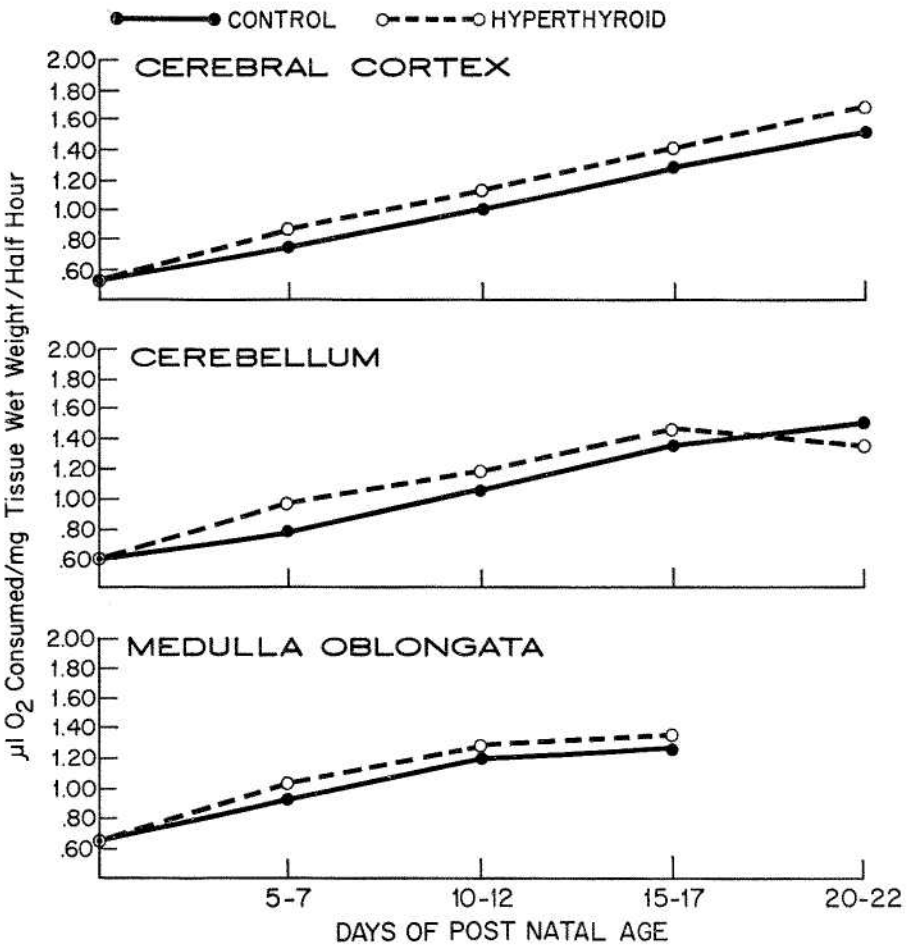


Fig. 2 Oxygen consumption of normal and hyperthyroid brain.

tissue (Hoexter, '54; Fazekas, Groves and Alman, '51).

While it would appear that the administration of excess thyroxin accelerated to a slight extent the "metabolic maturation" of brain tissue as measured by rates of O_2 consumption of glucose by brain slices, it is of interest that this hormonal effect was apparent only during the first week of postnatal life. In ten day old animals the O_2 consumption of brain did not differ significantly in hyperthyroid young rats from those of untreated controls anymore, at least in the areas tested.

A delay was also observed, with respect to rate of increase in dry weight of maturing cerebral and cerebellar cortex in hypothyroid rats. Changes in dry weight/wet weight ratio during development may reflect merely loss of water or increased protein synthesis, both of which may be influenced by thyroid hormone in the growing organism (Weil, '41). Although increase in dry weight in these two brain areas was considerably slowed down and delayed in the hypothyroid rat, normal adult levels approaching those of the controls were eventually attained in spite of the continued unavailability of the hormone to the growing animal.

Deprivation of thyroid hormone during early postnatal life resulted in more lasting effects with respect to maturation of conditioning behavior mechanism in the

TABLE 4

Dry weight determinations of cerebral cortex and cerebellum of normal and hypothyroid young rats
Mean % dry weight

	Age 10 days		Age 16–17 days		Age 23 days		Age 30 days		Age 40 days	
	(a) C	(b) Tx	(c) C	(d) Tx	(e) C	(f) Tx	(g) C	(h) Tx	(i) C	(j) Tx
Cerebral cortex (1)	11.5±0.53 (5)	11.1±0.66 (5)	16.1±0.17 (9)	11.5±0.26 (9)	—	—	15.4±0.22 (8)	12.2±0.32 (8)	20.9 (2)	18.2 (3)
Cerebellum (2)	11.6±0.30 (5)	10.1±0.66 (5)	16.2±0.45 (9)	12.2±0.33 (9)	14.0±0.22 (6)	11.9±0.37 (6)	16.1±0.54 (8)	13.5±0.17 (8)	21.6 (2)	21.2 (3)

C = control animals
Tx = hypothyroid animals.

The values are given ± the S.E.
The number of animals is represented in parenthesis.

Difference between Means

Groups	P	Significant difference
1a–1b	> 0.60	No
2a–2b	> 0.05	No
1c–1d	< 0.001	Yes
2c–2d	< 0.001	Yes
2e–2f	< 0.001	Yes
1g–1h	< 0.001	Yes
2g–2h	< 0.001	Yes

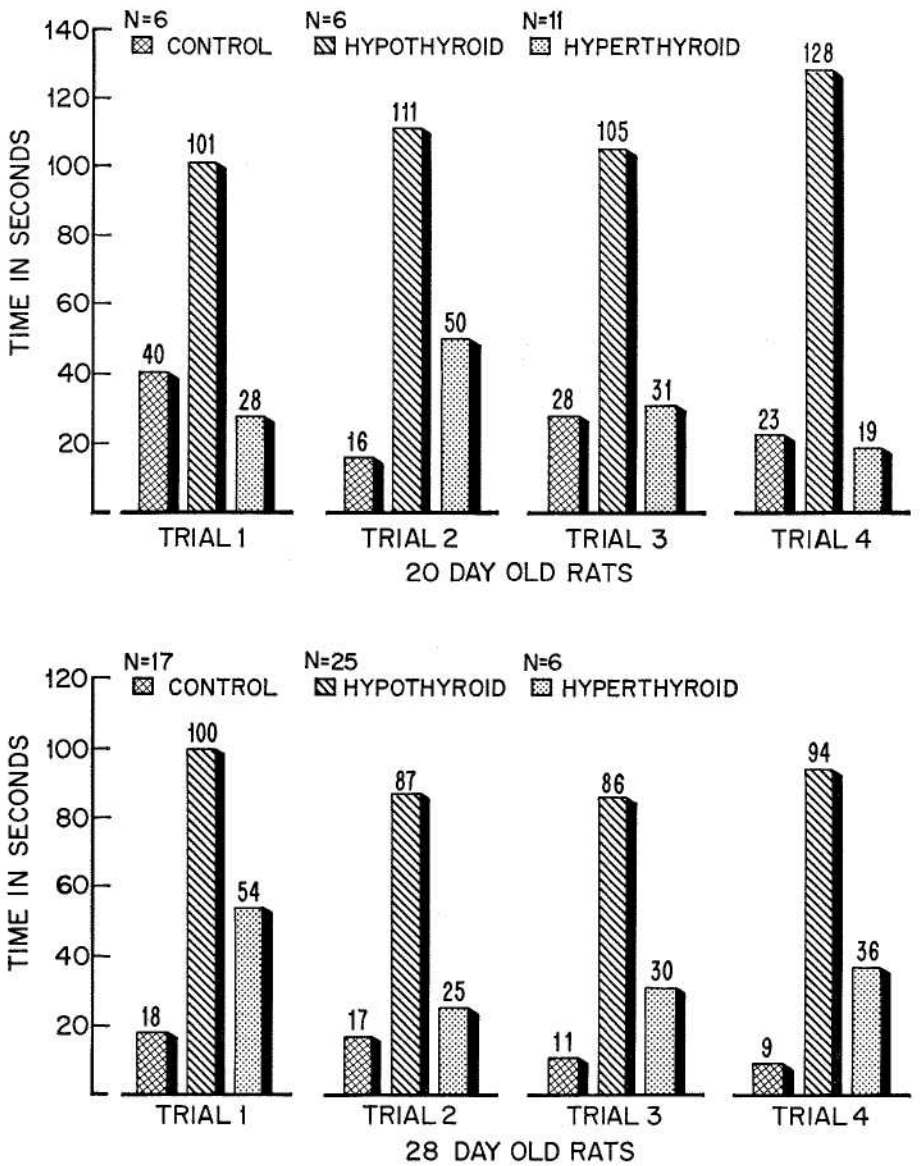
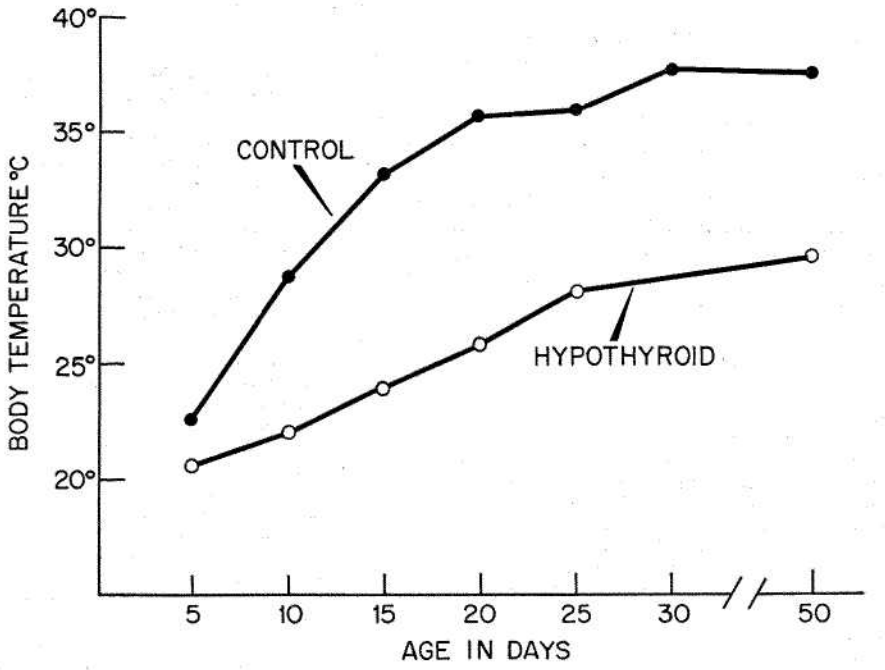


Fig. 3 Mean learning time of normal hypothyroid and hyperthyroid rats.

two groups. Using a water escape response test to measure "learning" it was noted that hypothyroid rats with few exceptions failed to "learn" in the four trials offered. This neagtive performance did not improve with increasing age. In one group of rats (not included in graph and table) maintained on PTU until day 50 the learning score was still zero. On the other hand, rats given excess thyroid hormone since birth attained the same level of achievement as untreated controls. Since no tests for "learning" performance were made prior to the twentieth day of age, we do not know, whether the emergence of this behavior can be advanced in hyperthyroid animals over that of untreated controls. In the light of the findings reported by



Rectal temperature of young rats. Each point represents the mean value of several determinations.

Fig. 4 Emergence of thermoregulation in normal and hypothyroid young rats.

Eayrs ('53, '54, '55, '59, '60), Eayrs and Horn ('55) and Eayrs and Taylor ('51) showing that thyroid deficiency arising during early stages of maturation of the rat gives rise to persistent but reversible abnormalities in the development of cerebral cortex prominent among which is a decrease in extent and complexity of neuropil and a consequent reduction in the probability of interaction between neurons, this is not unexpected or surprising. Irreversible enzymatic changes in the developing cerebral cortex of thyroidectomized rats were also noted by Hamburgh and Flexner ('57).

A persistent effect of thyroid deprivation on the maturation of the thermoregulating mechanism of the rat was also noted. Although in this study no effort has been made to distinguish between central or peripheral components of this mechanism the observations are arbitrarily included. The persistent failure of hypothyroid rats ever to reach adult levels of mammalian internal temperature if continually deprived of thyroid hormone from birth on, is probably related to their low survival rates in standard laboratory environment.

The diverse observations reported in this study can be balanced by assuming that in the rat the critical period during which hormone is required may vary for the maturation of different tissues and within the central nervous system even for different areas, but that prenatal development is relatively independent of availability of thyroxin at least for the parameters examined in this study. Possibly conditions prevailing *in utero* are so well "buffered" that the presence or absence of thyroid hormone is not a critical factor and consequently exerts little influence on developmental processes taking place during the prenatal period.

The first or the first two weeks of postnatal life on the other hand may constitute a period during which availability of thyroid hormone may be critical for growth and development processes to proceed at normal rates. Subsequent to this

"critical" period other rate controlling factors may emerge, enabling the hypothroid animals to catch up with the normal in most respects.

Our experimental results do not permit us to indentify the factors or combination of factors that operate in the uterine environment making the rat fetus independent of thyroid hormone, nor those that may emerge subsequently in the postnatally maturing rat to exert their influence on developmental rates.

LITERATURE CITED

Barnett, R. J. 1948 Some aspects of the experimental cretinlike animal. Thesis, Yale University, School of Medicine.

Bradley, P. B., J. T. Eayrs, M. B. Glass and R. W. Heath 1961 The maturational metabolic consequences of neonatal thyroidectomy upon the recruiting response in the rat. Electroenceph. clin. Neurophysiol., *13:* 577–586.

Chu, J. P. 1944 Influence of the thyroid gland on pituitary gonadotrophic activity in the rabbit. Endocrinol., *34:* 90–102.

Dawson, A. B. 1926 A note on the staining of skeleton of cleared specimens with alizarin red. Stain Tech., *1:* 123–124.

Davenport, C. B., and W. W. Swingle 1927 Effects of operations upon the thyroid glands of female mice on the growth of their offspring. J. Exp. Zool., *48:* 395–440.

Dempsey, E. W., and E. B. Astwood 1943 Determination of the rate of thyroid hormone secretion at various environmental temperatures. Endocrinol., *32:* 509–518.

Eayrs, J. T. 1953 Hormones and the maturation of the central nervous system with special reference to the rat. British J. Animal Behaviour, *1:* 144.

——— 1954 The vascularity of the cerebral cortex in normal and cretinous rats. J. Anat., *88:* 164–173.

——— 1955 The cerebral cortex of normal and hypothyroid rats. Acta Anat., (Basel), *25:* 160–183.

——— 1959 The status of thyroid hormone in relation to the development of the nervous system. British J. Animal Behaviour, *7:* 1–17.

——— 1960 Influence of the thyroid on the central nervous system. Brit. med. Bull., *16:* 122–127.

Eayrs, J. T., and G. Horn 1955 The development of the cerebral cortex in hypothyroid and starved rats. Anat. Rec., *121:* 53–61.

Eayrs, J. T., and W. A. Lishman 1955 The maturation of behaviour in hypothyroidism and starvation. British J. Animal Behaviour, *3:* 17.

Eayrs, J. T., and S. H. Taylor 1951 Effect of thyroid deficiency induced by methyl thiouracil on the maturation of the central nervous system. J. Anat., *85:* 350–358.

Eleftheriou, B. E. 1962 The role of the thyroid gland during growth and maturation in two subspecies of Peromyscus maniculatus. Purdue University Thesis. University Microfilms, Inc., Ann Arbor, Mich.

Essman, W. B., and M. E. Jarvik 1961 A water escape test for mice. Psychol. Rep., *8:* 58.

Etkin, W. 1964 Metamorphosis. Chapter 8 in Physiology of Amphibia. ed. J. Moore. Acad. Press: pp. 427–468.

Fazekas, J. F., F. B. Graves and R. W. Alman 1951 The influence of the thyroid on cerebral metabolism. Endocrinol., *48:* 169–174.

Fell, H. B., and E. Mellanby 1955 The biological action of thyroxine on embryonic bones grown in tissue culture. J. Physiol., *127:* 427–447.

——— 1956 The effect of 1 triiodothyronine on the growth and development of embryonic chick limb bones in tissue culture. J. Physiol., *133:* 89–100.

Hamburgh, M. 1955 Enzyme activities in the developing nervous system of normal and hypothyroid rats. Anat. Rec., *121:* 304.

Hamburgh, M., and L. B. Flexner 1957 Biochemical and physiological differentiation during morphogenesis. XXI. Effect of hypothyroidism and hormone therapy on enzyme activities of the developing cerebral cortex of the rat. J. Neurochem., *1:* 279–288.

Hamburgh, M., and E. Vicari 1957 Effect of thyroid hormone on nervous system maturation. Anat. Rec., *127:* 302.

Hamburgh, M., E. Sobel, R. Koblin and A. Rhinestone 1962 The passage of thyroid hormone across the placenta in intact and hypophysectomized rats. Anat. Rec., *144:* 219–228.

Hammett, F. S. 1923a Thyroid Apparatus IX: Effect of loss of thyroid and parathyroid glands at 100 days of age on growth in body length, body weight, and tail length of male and female albino rats. Am. J. Physiol., *63:* 218–244.

——— 1923b Thyroid studies XII: The effect of thyroparathyroidectomy and parathyroidectomy at 100 days on the growth of the brain and cord of male and female rats. J. Comp. Neur., *35:* 313–335.

——— 1924 Studies of the thyroid apparatus XX: Effect of thyroparathyroidectomy and parathyroidectomy at 75 days of age on the growth of the brain and spinal cord of male and female albino rats. J. Comp. Neur., *37:* 15–30.

——— 1926a Studies of the thyroid apparatus XXIX: The role of the thyroid apparatus in growth. Am. J. Physiol., *76:* 69–91.

——— 1926b Thyroid apparatus XXX: Relation between age at initiation of and response of body growth to thyroid deficiency. Endocrinol., *10:* 29–42.

——— 1926c Studies of the thyroid apparatus XXXI: Role of the thyroid and parathyroids in growth of the central nervous system. J. Comp. Neur., *41:* 171–203.

——— 1926d Studies of the thyroid apparatus XXXII: The role of the thyroid in solids-water differentiation of the nervous system during growth. J. Comp. Neur., *41:* 205–222.

Hodges, R. E., H. E. Hamilton and W. C. Keettel 1952 Pregnancy in myxedema. Arch. of Internal Med., *90:* 863–868.

Hoexter, F. M. 1954 The effect of thyroidectomy on the O_2 uptake of brain cortex and liver in the rat. Endocrinol., *54:* 1–4.

Horn, G. 1955 Thyroid deficiency and variation: the effects of replacement therapy on the development of the cerebral cortex of young albino rats. Anat. Rec., *121:* 63–79.

Hughes, A. A. 1944 Cretinism in rats induced by thiouracil. Endocrinol., *34:* 69–76.

Hunter, M. W., and P. B. Sawin 1942 The effect of thyroidectomy on the skull of the domestic rabbit. Am. J. Anat., *71:* 417–449.

Kollross, J. 1942 Localized maturation of lid closure reflex mechanism by thyroid implants into tadpole hindbrain. Proc. Soc. Exp. Biol. and Med., *49:* 204–206.

Kollross, J., and V. Peppernits 1952 Hormonal control of the size of mesencephalic V nucleus cells in Rana pipiens. Anat. Rec., *113:* 527.

Krohn, P. L., and H. C. White 1950 The effect of hypothyroidism on reproduction in the female albino rat. J. of Endocrinol., *6:* 375–385.

Legrand, T., A. Kriegel and A. Jost 1961 Deficience thyroidienne et maturation du cervelet chez le rat blanc. Arch. d'Anatomie Microscopique et de Morphol., *50:* 507–519.

Legrand, T. 1963 Maturation du cervelet et deficience thyroidienne donnees chronologiques. Arch. d'Anatomie Microscopique et de Morphol. Experiment, *52:* 205–214.

Liddell, H. S. 1923a Methods of investigating the effect of thyroidectomy on neuromuscular mechanisms of sheep. Quart. J. Exp. Physiol., *13:* 191.

——— 1923b Possible influence of fatique on the reaction time of thyroidectomized sheep. Proc. Soc. Exp. Biol. and Med., *21:* 126–127.

——— 1926 Effect of thyroidectomy on some unconditioned responses in sheep and goats. Am. J. Physiol., *75:* 579–590.

——— 1927 Higher nervous activity in the thyroidectomized sheep and goat. Quart. J. Exp. Physiol., *17:* 41.

Liddell, H. S., and E. D. Simpson 1926 A preliminary study of conditioned motor reflexes in thyroidectomized sheep. Proc. Soc. Exp. Biol. and Med., *23:* 720–722.

Liddell, H. S., and S. Simpson 1923 Neuromuscular activity in cretinoid sheep. Am. J. Physiol., *63:* 406.

Liddell, H. S., W. T. Jones and O. D. Anderson 1934 The comparative physiology of the conditioned motor reflex. Comp. Psychol. Monogr., *11:* 1.

Moore, C. R. 1950 The role of the fetal endocrine glands in development. J. Clin. Endocrinol., *10:* 942–985.

Osorio, C., and N. B. Myant 1960 The passage of thyroid hormone from mother to foetus and its relation to foetal development. Brit. Med. Bull., *16:* 159–164.

Parkin, G., and J. A. Greene 1943 Pregnancy occurring in cretinism and adult myxedema. J. Clin. Endocrinol., *3:* 466–468.

Pitt-Rivers, R. and J. R. Tata 1959 Physiological actions of thyroid hormones. In: The Thyroid Hormones, 59–98 (Chapt. 5).

Reiss, J., M. Reiss and A. Wyatt 1956 Action of thyroid hormone on brain metabolism of newborn rats. Proc. Soc. Exp. Biol. and Med., *93:* 19–22.

Smith, E. E., and F. C. McLean 1938 Effect of hyperthyroidism upon growth and chemical composition of bone. Endocrinol., *23:* 546–552.

Todd, T. W., R. E. Wharton and A. W. Todd 1938 The effect of thyroid deficiency upon bodily growth and skeletal maturation in the sheep. Am. J. Anat., *63:* 37–78.

Weil, A. 1941 The chemical constitution of brains, kidneys and heart of white rats in experimental hypothyroidism. Endocrinol., *29:* 919–926.

Weiss, P., and F. Rosetti 1951 Growth response of opposite sign among different neuron types exposed to thyroid hormone. Proc. Nat. Acad. Sci. (U.S.A.), *37:* 540–556.

Weiss, R. M., and C. R. Noback 1949 The effect of thyroxin and thiouracil on the time of appearance of ossification centers of rat fetuses. Endocrinol., *45:* 389–395.

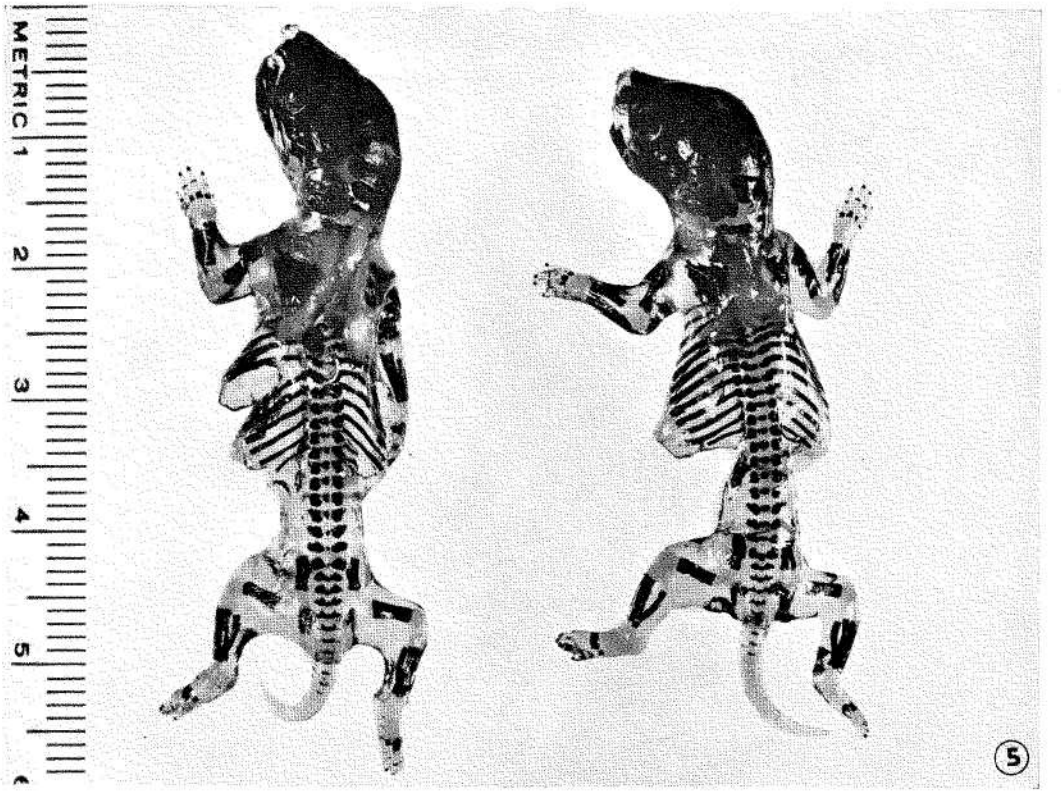
PLATE 1

EXPLANATION OF FIGURES

5 Skeleton of newborn rats. To the left is a newborn obtained from a normal untreated mother. To the right is a newborn obtained from a mother treated with propylthiouracil since the fifteenth day of gestation.
No differences in skeletal ossification are apparent. × 3.

6 Section through cerebellum of a 21 day old normal rat. The external granular layer has disappeared and the typical adult cerebellar architecture is established. × 120.

7 Section through cerebellum of a 21 day old hypothyroid rat. The external granular zone is still present as a multicellular layer. Extensive cell proliferation and migration of cells through the molecular zone is still visible. × 120.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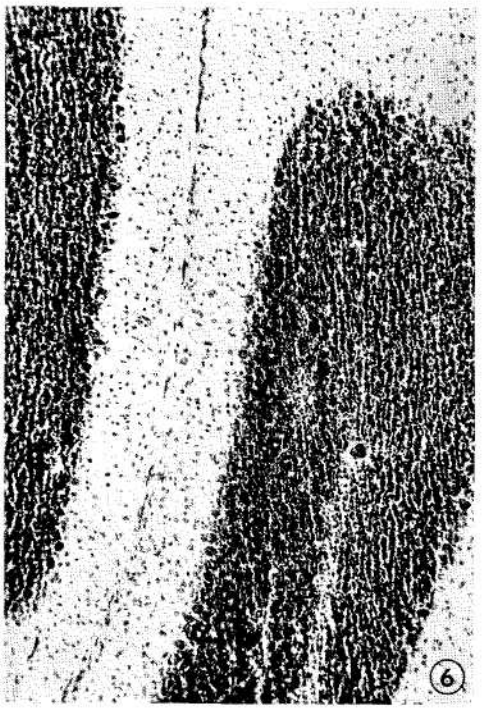
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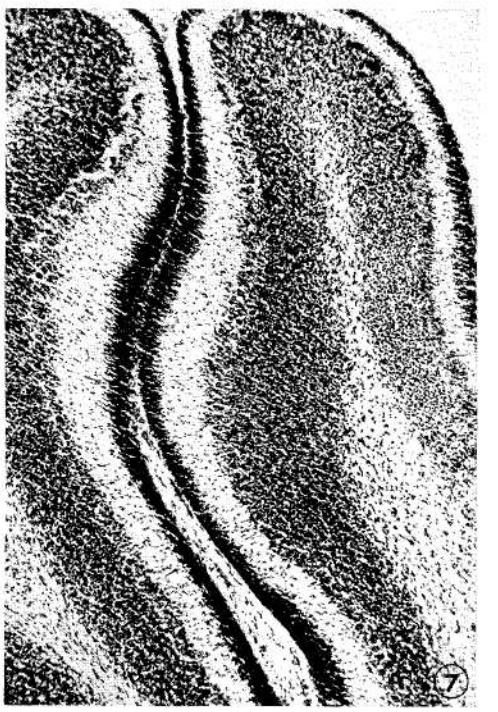
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