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HORMONAL EFFECTS ON THE DEVELOPMENT OF RAT BRAIN GANGLIOSIDES—II. THYROXINE*

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Abstract—Rats were given thyroxine (~ 1 μg/g of body weight) on days 2–4 of life. After this, endogenous ganglioside levels and incorporation of radionuclide from 1-[^{14}C]glucosamine were determined at various ages up to 3 weeks. Thyroxine caused an initial stimulation of ganglioside development, maximal at 1 week of age, followed by a diminution in production, resulting in decreased ganglioside levels at 20 days. The brainstem was affected least and the cerebellum most severely. Of seven resorcinol-positive materials studied individually, the initial stimulation was particularly noticeable in G_{M_1}, $G_{D_{1a}}$ and G_{T_1}, with $G_{D_{1b}}$ in some cases decreased. The ganglioside pattern at 20 days showed an abnormal preponderance of the less polar compounds.

The ability of gangliosides to bind and inactivate various toxins has been a topic of investigation for a number of years [1–3]. Only recently, however, has the possible role of gangliosides as receptors for endogenous hormones been studied. Recently, Mullin *et al.* [4] demonstrated the interaction of gangliosides with thyrotropin. They showed that the gangliosides differed in their ability to bind thyrotropin, with $G_{D_{1b}}$§ having the highest affinity, suggesting that this is not a nonspecific effect. The role of the thyroid axis in development has long been known in relation to such specific developmental events as metamorphosis [6] and to developmental aberrations such as congenital hypothyroidism (cretinism).

Schapiro [7, 8] carried out several studies of the effects of neonatal hormone administration on the development of rats. He reported that neonatal thyroxine administration or thyroidectomy did not affect the metabolic rate or body temperature of rats in the first 2 weeks of life, although rats essentially are poikilothermic in this interval. Thyroxine increased brain cholesterol and locomotor activity and accelerated eye opening. In other studies [9, 10], 1 μg/g body weight of thyroxine i.p. at days 1–3 of age, increased corticosterone levels in response to electric shock, a response not developing until later in control animals. EEG activation in response to stimuli also occurred earlier in treated animals, as did the startle response to loud noise and the emergence of swimming behavior. Another finding noted in treated animals was the more rapid acquisition of a conditioned avoidance response at 16–18 days of age. However, this initial potentiation of learning behavior may be reversed at later times. As adults, the animals of the thyroxine group made more errors than controls in a maze-learning situation [8].

Balazs *et al.* [11] studied rats receiving 25 μg triiodothyronine on the day of birth and 0·5 to 1.5 μg on alternate days thereafter. The experimental animals opened their eyes 3–4 days earlier than the control animals and were more active, but demonstrated reduced growth: body weight was 23 per cent lower than in control animals at 50 days of age, while significant reductions also occurred in cerebral weights by 5 days, and in cerebellar weights at a later time. Triiodothyronine reduced the increase in cell number during the rapid growth phase of both cerebrum and cerebellum, the eventual deficits being 15 and 30 per cent respectively.

These findings are in accord with other data indicating that both hypothyroid and hyperthyroid states have an adverse effect on central nervous system development [12, 13]. Thyroxine is thought to cause a premature differentiation of cells, giving at first accelerated development, but finally a decreased cell number due to early cessation of proliferation. Schapiro's findings as to swimming behavior have been confirmed in our laboratory [14], and thyroxine has been found also to accelerate the course of post-natal changes in levels of activity of ornithine decarboxylase [15]. As part of a larger project, experiments were performed to measure the effect of neonatal thyroxine administration upon the gangliosides of developing rat brain. Whole brains and three brain regions were studied, since gangliosides differ in various brain areas and during development [16].

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§ The nomenclature of Svennerholm [5] is used throughout.

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MATERIALS AND METHODS

All animals, materials and procedures were exactly as described in the companion paper [17]. Sodium levothyroxine (Synthroid) was obtained from Flint Laboratories, Morton Grove, IL; it was dissolved in distilled water to a concentration yielding approximately 1 μg/g of body weight in a volume of 10 μl based on the average body weight of all thyroxine-treated animals. Each animal received 7.7 μg thyroxine, i.p., on day 2 of life, 8.6 μg on day 3, and 9.5 μg on day 4. Unused thyroxine was discarded at the end of the day. [^{14}C]glucosamine (NEC 193X, New England Nuclear Corp., Boston, MA) was injected subdurally 24 hr prior to decapitation. *In vivo*, this has been shown to label primarily the *N*-acetylneuraminic acid (NANA) residues [17]. Animals were decapitated and the brain was removed within 1 min. When appropriate, dissection was carried out into cerebral hemispheres (cortex, basal ganglia and associated white matter), cerebellum and brainstem (the remainder). Parts were pooled and gangliosides prepared by a modification of the method of Suzuki [16]. Where indicated, chromatography was performed in chloroform–methanol–2.5 N NH_4OH (55:45:9). The resulting chromatograms yielded seven resorcinol-positive spots, of which spots # 2, 3, 4, 6 and 7 corresponded (by comparison) with G_Q, G_{T_1}, $G_{D_{1b}}$, $G_{D_{1a}}$ and G_{M_1} of Svennerholm [5] respectively. NANA was determined by a modification of the periodate–resorcinol method of Jourdian *et al.* [18] and radioactivity was counted in a Nuclear-Chicago Mark II liquid scintillation spectrometer and converted to disintegrations per minute (dis./min).

Endogenous ganglioside levels were measured in whole brains at 6, 7, 9, 10, 11, 13, 15, 16 and 20 days and in cerebral hemispheres, brainstem and cerebellum at 6, 7, 10, 11, 15, 16 and 20 days. Incorporation of label from [^{14}C]glucosamine and distribution of NANA among chromatographic spots were measured at 6, 10, 15 and 20 days for whole brain and at 6, 10 and 20 days for brain parts. Altogether, 70 whole brains and 144 dissected brains were studied after thyroxine treatment, while 79 whole brains and 80 dissected brains served as controls. The controls did not differ from larger groups of normally developing rats.

RESULTS

Growth. Injection of sodium levothyroxine on days 2–4 of life exerted a biphasic effect on the growth of rats. There was facilitation of growth, in terms of body weight, beginning with the initiation of drug treatment and enduring for 3 days. The maximum departure from controls was plus 11 per cent on day 2 of treatment ($P < 0.001$). By day 5, the body weights of treated animals had returned to normal, but by 10 days of age they had fallen to 88 per cent of control ($P < 0.001$) and remained at about the 90 per cent mark through day 20. Mortality of thyroxine-treated animals over the first 6 post-natal days was 85 per cent of untreated controls.

Whole brain showed only a nonsignificant increase in weight at 5 days, but by 11 days of age weighed 17 per cent less than in controls ($P < 0.001$) and,

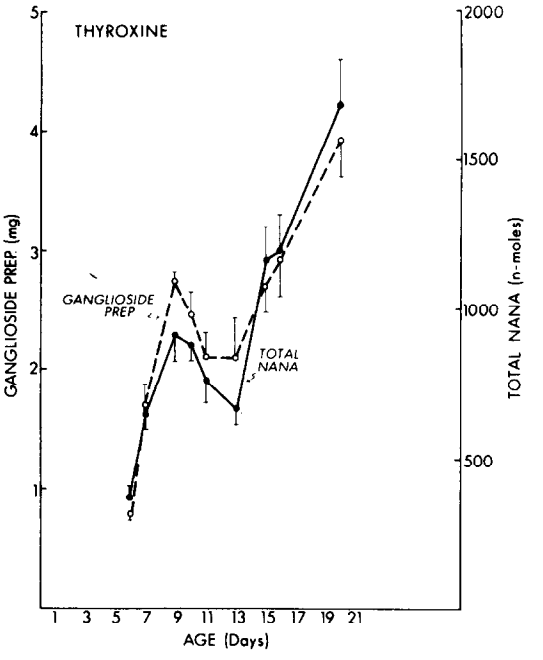

Fig. 1. Weight of ganglioside preparation and value of its NANA content in whole brains of rats given 1 μg/g body weight of thyroxine i.p. at 2–4 days of age. Rats of the ages indicated were decapitated and the brains removed rapidly. Gangliosides were prepared as described in the text and NANA content was determined by the periodate–resorcinol method.

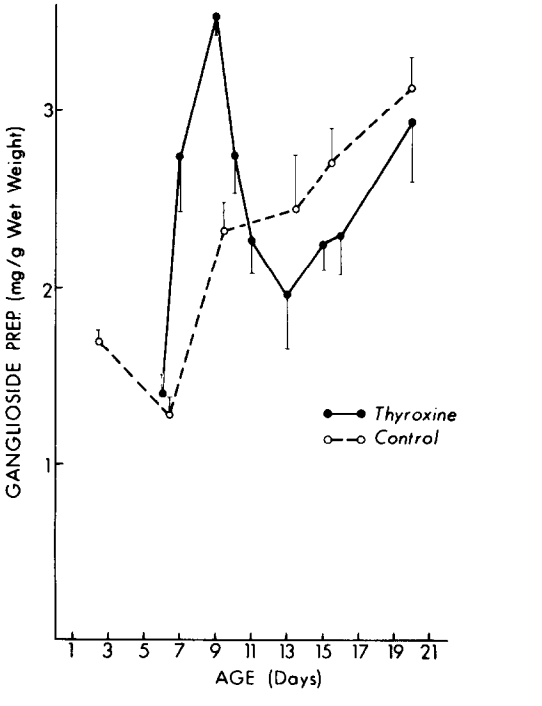

Fig. 2. Weight of ganglioside preparation per gram wet weight of brain tissue from rats given 1 μg/g body weight of thyroxine i.p. at 2–4 days of age and from controls. Rats of the ages indicated were decapitated and the brains removed rapidly. Gangliosides were prepared as described in the text.

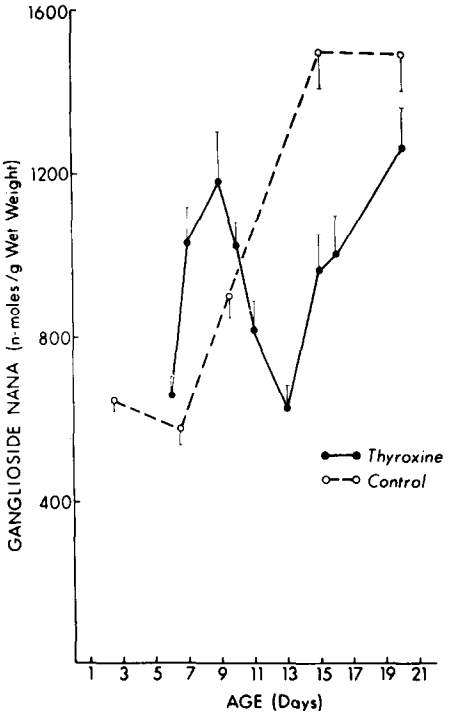

Fig. 3. NANA content of ganglioside preparation per gram wet weight of brain tissue from rats given 1 μg/g body weight of thyroxine i.p. at 2–4 days of age from controls. Rats of the ages indicated were decapitated and the brains removed rapidly. Gangliosides were prepared as described in the text, and NANA content was determined by the periodate–resorcinol method.

although showing some tendency to recover, was still less at 20 days of age (95 per cent, $P < 0.05$), when the study was concluded. The increased weight of the cerebrium at 6 days was significant (115 per cent, $P < 0.05$) and the values after that followed the course of whole brain very closely. Brainstem weight was not affected significantly in any respect by neonatal thyroxine treatment. The cerebellum was affected differentially, being decreased in weight at 6 days (83 per cent, $P < 0.001$) when the cerebrum weight was increased (v.s.), and at 7 days (88 per cent, $P < 0.001$), then recovering to a normal weight at 10 and 11 days (101 and 105 per cent), and falling again finally at 15–16 days (88 per cent, $P = 0.06$) and at 20 days (86 per cent, $P < 0.001$).

Endogenous total ganglioside. Ganglioside content was measured in terms of both weight of preparation and NANA content. As with controls, the curves followed each other closely (see Fig. 1). The results are compared to those of controls on the basis of tissue wet weight, in Figs. 2 and 3. Again, a biphasic effect was noted. During the period from 6 to 9 days, when normal animals showed a rapid accumulation of ganglioside, the brains of thyroxine-treated rats showed an even greater gain. This was significant at 7 and 9 days of age ($P < 0.001$). Beginning at 9 days, however, the ganglioside content in the experimental group actually declined and the NANA concentration at 13 days returned to its 6-day value. This was significantly below controls in terms of sialic acid (49 per cent, $P < 0.001$), but not in terms of the weight of the ganglioside preparation (70 per cent). This disjunction roughly suggests an alteration in the ganglioside pattern (v.i.). From 13 days on, the ganglioside content showed a rapid rise, reaching 94 per cent of control for the ganglioside preparation/g of tissue, but only 84 per cent for NANA/g weight by 20 days ($P < 0.001$).

The study of brain parts indicated that the thyroxine acted differently in various areas of the brain (see Table 1). The cerebrum and brainstem showed only depression of ganglioside content in response to thyroxine, the onset being earlier in the brainstem. There was a tendency toward recovery, also manifested earlier in brainstem. The cerebellum responded with early stimulation to thyroxine, achieving a ganglioside level of about five times control at 7 days by the ganglioside preparation and about three times control by NANA. The level then dropped acutely although a disjunction between NANA and ganglioside weight suggested an alteration in the distribution of NANA among the gangliosides.

Total ganglioside radionuclide incorporation. The incorporation of radioactivity from intracerebrally injected [^{14}C]glucosamine into the brains of thyroxine-treated animals differed significantly from controls (see Table 2). The difference for whole brain, as with endogenous NANA content, was biphasic.

At 6 days of age, the radioactivity of experimental brains was nearly twice as great as controls, and this was significant in all brain regions, being most marked in cerebellism. However, by 10 days of age, whole brain ganglioside labeling was only slightly above control, and significantly elevated only in brainstem. Whole brain incorporation remained

Table 1. Total ganglioside NANA in brain regions of thyroxine-treated vs control rats*

Per cent of control—per part (per g)							
	6	7	10	11	15	16	20
Cerebral Hemispheres	90 (78)	85 (92)	80 (93)	56† (63)†	45‡ (45)‡	79† (69)†	75 (69)
Brainstem	95 (52)	71§ (73)§	90 (101)	89 (98)	75§ (75)§	79 (75)	88 (93)
Cerebellum	123 (89)	307‡(261)‖	66‡ (57)‡	57‡ (64)‖	61 (68)§	61‖ (71)†	65‖ (51)‡

* Experimental animals received 1 μg/g body weight of thyroxine i.p. at 2–4 days of age. Each value represents six to twelve assays.

† $P < 0.01$.

‡ $P < 0.001$.

§ $P < 0.05$.

‖ $P < 0.005$.

Table 2. Incorporation of label from intracerebrally injected [^{14}C]glucosamine into brain ganglioside of thyroxine-treated rats during development*

	Age (days)			
	6	10	15	20
Whole brain				
(dis./min/brain/μCi injected)	173,411 ± 13,253	127,943 ± 11,359	60,007 ± 5,109	27,865 ± 2,431
(% Control)	197†	105‡	91‡	61†
Cerebral hemispheres				
(dis./min/part/μCi injected)	106,770 ± 10,000	75,464 ± 517	×	19,320 ± 1,841
(% Control)	191†	91‡	×	79‡
Brainstem				
(dis./min/part/μCi injected)	57,156 ± 5,462	39,195 ± 3,981	×	5,381 ± 679
(% Control)	188†	141§	×	40†
Cerebellum				
(dis./min/part/μCi injected)	6,928 ± 1,252	6,630 ± 889	×	2,033 ± 308
(% Control)	220§	127‡	×	37‖

* Experimental animals received 1 μg/g body weight of thyroxine at 2–4 days of age. Each value represents 12–29 separate assays for whole brain and 9–17 assays for brain regions.
† $P < 0.001$.
‡ Not significant.
§ $P < 0.05$.
‖ $P < 0.005$.

normal at 15 days, but fell significantly below control levels at 20 days, when endogenous NANA correspondingly was low (v.s.). This decrease relative to controls was not significant in the cerebral hemispheres, but was highly so in brainstem and cerebellum.

Developmental course of seven individual resorcinol-positive materials. Data pertaining to the NANA content of individual chromatographic areas are compared with control values in Table 3. At day 6, an increase in total NANA content of whole brain was significant in spot #7; spot #5 was somewhat decreased. The cerebral hemispheres showed no significant differences from the controls in the ganglioside pattern at this stage of development. Brainstem had a reduced amount of NANA in spot #4, but an increased amount and proportion of spot #7. Cerebellum showed a shifted ganglioside pattern, with spots #3 and 6 increased in their percentage of the total (160 per cent, $P < 0.05$ and 166 per cent, $P < 0.025$) and spots #1, 4 and 5 decreased (22 per cent, $P < 0.001$, 40 per cent, $P < 0.005$ and 40 per cent, $P < 0.01$).

At 10 days, the peak of ganglioside increase over controls for whole brain had passed. At this time spots #1 and 4 were found to be decreased slightly, and spot #7 was increased by about one-fourth the control value, although this failed to achieve statistical significance. When brain parts were considered separately, the cerebrum again did not differ from controls. The brainstem too was not unlike controls, except for a continued elevation in both content and proportion of spot #7 NANA (177 per cent of total, $P < 0.005$). In cerebellum, two of the three spots which had been diminished in their percentage at 6 days remained below normal, #4 (21 per cent, $P < 0.001$) and #5 (52 per cent, $P < 0.05$). Those which had been elevated (#3 and 6) returned to control level.

At 15 days, total ganglioside NANA was decreased relative to controls (v.s.) and this was apparent in all spots except #7. The disjunction noted earlier between weight of the ganglioside preparation and NANA content is thus explicable as an increase in monosialoganglioside, spot #7.

At 20 days, the total ganglioside is still decreased, and this is significant in spots #1–4. Spots #1 and 5 are diminished significantly in their percentage of the total (46 per cent, $P < 0.01$ and 52 per cent, $P < 0.001$) while spot #7 is increased significantly (172 per cent, $P < 0.0025$). In the cerebral hemispheres, for the first time, spots #1, 3 and 4 were depressed in NANA content and there was a shift in ganglioside pattern, with the above three spots being decreased in percentage and spot #7 now being increased (162 per cent, $P < 0.025$). In the brainstem, where spot #7 had been high consistently, it returned to a level slightly, but no longer significantly, above control level. Spot #4, which had been somewhat decreased previously, was significantly so at 20 days and spot #6 was increased slightly as a percentage of the total (137 per cent, $P < 0.05$). The decrease in cerebellar NANA was manifest in all spots except #5 and 7, while the ganglioside pattern was shifted sharply toward #7 (254 per cent, $P < 0.005$) at the expense primarily of #1 (59 per cent, $P < 0.05$) and #2 (51 per cent, $P < 0.025$).

The distribution of radioactivity among the seven chromatographic spots in thyroxine-treated brains is compared with the control level in Table 4. At 6 days, increased incorporation was manifest in all spots, but there was a percentage shift in pattern away from spot #3, the major trisialoganglioside and toward spot #7, the major monosialoganglioside. This was true equally when cerebrum was measured separately, where the proportion of counts contained in spot #5 was also increased. In brainstem, also, all spots showed increased incorporation, but the proportion of spot #7 was not changed from controls. The cerebellum, which showed the greatest stimulation at 6 days, also had the most apparent distortions in the pattern of incorporation. Spot #4 incorporated only 29 per cent of its control percentage of the total dis./min ($P < 0.001$)

Table 3. Brain ganglioside NANA of thyroxine-treated vs control rats*

Spot	NANA (nmoles as per cent of control) Age (days)			
	4	10	15	20
Whole Brain				
# 1	91†	62‡	27‡	37‡
# 2 (G_Q)	100†	82†	57‡	68§
# 3 (G_{T_1})	122†	105†	72‡	65‖
# 4 ($G_{D_{1b}}$)	83†	66†	44¶	42‡
# 5	69¶	70†	79†	82†
# 6 ($G_{D_{1a}}$)	104†	94†	81‡	104†
# 7 (G_{M_1})	137§	122†	98†	138†
Cerebral hemispheres				
# 1	133†	83†	×	38‡
# 2 (G_Q)	100†	135†	×	92†
# 3 (G_{T_1})	133†	102†	×	53‖
# 4 ($G_{D_{1b}}$)	115†	94†	×	26‡
# 5	71†	89†	×	86†
# 6 ($G_{D_{1a}}$)	119†	88†	×	101†
# 7 G_{M_1})	110†	91†	×	127†
Brainstem				
# 1	33†	63†	×	81†
# 2 (G_Q)	120†	83†	×	62†
# 3 (G_{T_1})	73†	117†	×	73†
# 4 ($G_{D_{1b}}$)	50§	77†	×	44‡
# 5	140†	94†	×	64†
# 6 ($G_{D_{1b}}$)	79†	104†	×	113†
# 7 (G_{M_1})	173§	190‖	×	133†
Cerebellum				
# 1	40†	50†	×	50§
# 2 (G_Q)	100†	50†	×	33¶
# 3 (G_{R_1})	267†	75†	×	52§
# 4 ($G_{D_{1b}}$)	75†	10‡	×	43§
# 5	75†	38¶	×	81†
# 6 ($G_{D_{1a}}$)	260§	60†	×	69§
# 7 (G_{M_1})	163†	58¶	×	158†

* Experimental animals received 1 μg/g of thyroxine i.p. at 2–4 days of age.
† Not significant.
‡ $P < 0.001$.
§ $P < 0.05$.
‖ $P < 0.005$.
¶ $P < 0.01$.

and actually was decreased in uptake from controls (60 per cent, $P < 0.025$). Spot # 1 accounted for only 50 per cent of its control percentage of the total ($P < 0.001$). All other spots were increased in their actual incorporation. Spots # 3 and 7 were proportionally the most augmented (129 per cent, $P < 0.05$ and 171 per cent, $P < 0.001$), while spot # 6 was increased slightly, but not significantly.

In whole brain at 10 days of age, although total label was equivalent to controls, the incorporation into spot # 7 continued to be greater than normal. Spot # 3 was decreased and spot # 1 was increased. Cerebrum showed alterations in its pattern, with spots # 2 and 5 taking up label at about one-half the control level, while spot # 3 was also decreased significantly. Spot # 1 was increased slightly and spot # 7 was about triple the control values in terms of radioactivity (282 per cent, $P < 0.001$) and percentage of the total label (308 per cent, $P < 0.001$). Brainstem, which showed greatest overall stimulation at this age, had a normal pattern, except for a moderate decrease in spot # 4 and a moderate increase in spot # 7. In cerebellum, where the overall incorporation was down nearly to control level, spot # 4 remained diminished in percentage and spot # 7 continued to be increased in accumulation of label.

Brains of 15-day-old rats, which were normal in overall incorporation, remained elevated in spot # 7 (229 per cent, $P < 0.001$). In addition, spot # 2 incorporation was high at this point (172 per cent, $P < 0.05$). This was balanced by significant decrease in the labeling of spots # 3 (74 per cent, $P < 0.05$) and # 4 (48 per cent, $P < 0.005$).

At 20 days, the significant decrease in incorporation relative to controls was seen in all spots except # 1 and 7. In terms of distribution, spot # 3 remained decreased

Table 4. Radioactive labeling of brain gangliosides of thyroxine-treated vs control rats*

Spot	Proportion of total dis./min (per cent control) Age (days)			
	6	10	15	20
Whole brain				
# 1	121†	133†	100†	126†
# 2 (G_Q)	121†	76†	193‡	95†
# 3 (G_{T_1})	81§	77‡	82†	81‖
# 4 ($G_{D_{1b}}$)	94†	90†	51¶	96†
# 5	118†	75†	85†	91†
# 6 ($G_{D_{1a}}$)	98†	100†	100†	98†
# 7 (G_{M_1})	141¶	269¶	256¶	183‖
Cerebral hemispheres				
# 1	180†	153†	×	135†
# 2 (G_Q)	83†	56‖	×	60†
# 3 (G_{T_1})	84†	80‖	×	80†
# 4 ($G_{D_{1b}}$)	124†	92†	×	96†
# 5	186§	57¶	×	118†
# 6 ($G_{D_{1a}}$)	94†	97†	×	104†
# 7 (G_{M_1})	99†	168‡	×	278¶
Brainstem				
# 1	86†	119†	×	187†
# 2 (G_Q)	237‖	76†	×	108†
# 3 (G_{T_1})	92†	100†	×	88†
# 4 ($G_{D_{1b}}$)	93†	58¶	×	84†
# 5	79†	82†	×	98†
# 6 ($G_{D_{1a}}$)	110†	90†	×	99†
# 7 (G_{M_1})	99†	168‡	×	278¶
Cerebellum				
# 1	92†	133†	×	133†
# 2 (G_Q)	50¶	79†	×	74‡
# 3 (G_{T_1})	129‡	111†	×	93†
# 4 ($G_{D_{1b}}$)	29¶	56‖	×	99†
# 5	74†	72†	×	74‡
# 6 ($G_{D_{1a}}$)	120†	127†	×	97†
# 7 (G_{M_1})	120¶	146†	×	202‡

* Experimental animals received 1 μg/g body weight of thyroxine i.p. at 2–4 days of age.
† Not significant.
‡ $P < 0.05$.
§ $P < 0.01$.
‖ $P < 0.025$.
¶ $P < 0.001$.

and spot # 7 remained increased. Cerebral hemispheres, which were decreased least overall, evinced a pattern of incorporation consistent with the whole brain pattern and with their own at 10 days. In brainstem, the elevation of spot # 7 was the only significant alteration in pattern of distribution, accounting for 278 per cent of the control percentage of total incorporation ($P < 0.001$). In cerebellum the diminution of uptake of radioactivity was so severe that not even spot # 7 escaped being decreased (77 per cent, $P < 0.0025$), although it was affected least of all. In distribution, spots # 2 and 4 were decreased somewhat and spot # 7 was double the control percentage.

DISCUSSION

Thyroid hormones, given to the neonate, appear to accelerate biochemical and behavioral maturation during early development [7, 10, 13]. However, they may have long-range adverse effects on growth and central nervous system function [8, 11, 13]. These data may be explained by the hypothesis that the hormone acts to stimulate maturation and differentiation of neuronal elements, with the immediate consequence of neurochemical and behavioral precocity and an ensuing premature halt in the proliferative stage of brain development with reduction in ultimate cell number and functional capacity [12].

All findings of the present study are in accord with this hypothesis. The data suggest that the extent of influence of thyroxine was related to the activity and type of development occurring in a particular brain part.

The ganglioside content of whole brains from animals treated with thyroxine showed a clear biphasic hormone effect. The inflection point was located at about 10 days of age, with the stimulatory phase peaking at 7 days and the maximum depression occurring at about 13 days. Ganglioside NANA was still significantly below normal at 20 days. This developmental pattern is reflected in the incorporation of label from [^{14}C]glucosamine. It should be noted that there was an actual decrease in NANA content between 9 and 13 days of age. It may be that after a period of drug-induced hyperactive synthesis (as reflected in increased incorporation of label at 6 days), there is a rebound phenomenon and degradative processes predominant for a time. Although radionuclide is incorporated at a normal rate between 9 and 10 days, this might reflect exchange with the abnormally increased ganglioside pool at this stage, rather than a normal rate of synthesis.

The differences among brain regions in the response of gangliosides to thyroxine treatment are great. The increase in ganglioside content at 6–7 days of age was found almost entirely in the cerebellum. At 7 days of age, 4.6 times the normal weight of ganglioside was prepared from this region and it possessed three times the NANA of controls. Synthesis must have been stimulated greatly during the period of hormone administration, for at 6 days, cerebellar incorporation of ^{14}C-label was still more than double the control level. The severe depression of cerebellar NANA content and incorporation of label in weeks 2 and 3 after drug treatment had not recovered by the end of the study. Thyroxine, then, had a generally negative effect on total brain gangliosides. Brainstem was able to recover almost completely by 20 days of age, cerebral hemispheres had recovered to 80 per cent of control levels, while cerebellum did not recover and remained at half the normal concentration of NANA at 20 days. These results are in close accord with previous reports on the action of thyroxine upon brain development [12, 14, 19], i.e. an early commencement of development, an accelerated developmental period, and a premature cessation of development with consequent deficit later in life.

With respect to the four major brain gangliosides, G_{T_1} (spot # 3) and G_{M_1} (spot # 7) showed the greatest increases at 6 days of age, while at 20 days of age, the general decrease in gangliosides pertained most to $G_{D_{1b}}$ (spot # 4), next most to G_{T_1} (spot # 3) and not at all to $G_{D_{1a}}$ and G_{M_1} (spots # 6 and 7). The increase in incorporation of radionuclide into G_{M_1} (spot # 7) was prolonged beyond that of the other gangliosides and was still slightly elevated at 20 days of age.

As to the minor spots, spot # 5 was unusual in showing decreased NANA at 6 days, but the validity of this finding is questionable because of the small values involved. Spots # 1 and 2 were among the most severely decreased substances at 15 and 20 days. The radioactivity of spot # 2 showed peculiar characteristics, being extremely high relative to its sialic acid content at 15 days.

Regional differences in the patterns of individual gangliosides under the influence of thyroxine were similar to those for total ganglioside. Brainstem showed the deficit in $G_{D_{1b}}$ (spot # 4) earlier and sustained the increased sialic acid and labeling of G_{M_1} (spot # 7) longest, both consistent with its greater state of maturation.

The pattern of ganglioside distribution at the end of the thyroxine study bears a resemblance to that found at the same age after cortisol treatment [17]. It may be wondered whether this represents a ganglioside pattern associated with retarded development and a decreased level of total ganglioside.

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REFERENCES

1. W. E. Van Heyningen, *J. gen. Microbiol.* **20**, 310 (1959).
2. W. E. Van Heyningen and J. Mellanby, in *Microbial Toxins* (Eds. K. Solomon, T. C. Montie and S. J. Ajl), Vol. IIA, p. 69. Academic Press, New York (1971).
3. L. L. Simpson and M. M. Rapport, *J. Neurochem.* **18**, 1341 (1971).
4. B. R. Mullin, P. H. Fishman, G. Lee, S. M. Aloj, F. D. Ledley, R. J. Ninand, L. D. Kohn and R. O. Brady, *Proc. natn. Acad. Sci. U.S.A.* **73**, 842 (1976).
5. L. Svennerholm, *J. Neurochem.* **10**, 613 (1963).
6. E. B. Astwood, in *The Pharmacological Basis of Therapeutics*, 4th Edn (Eds. L. S. Goodman and A. Gilman), p. 1466. MacMillan, New York (1970).
7. S. Schapiro, *Endocrinology* **78**, 527 (1966).
8. S. Schapiro, *Gen. comp. Endocr.* **10**, 214 (1968).
9. S. Schapiro and R. J. Norman, *Science, N.Y.* **165**, 1279 (1967).

10. S. Schapiro, M. Salas and K. Vukovich, *Science, N.Y.* **168**, 147 (1970).
11. R. Balazs, S. Kovals, W. A. Cocks, A. L. Johnson and J. T. Eayrs, *Brain Res.* **25**, 555 (1971).
12. J. R. Eayrs and S. H. Taylor, *J. Anat.* **85**, 350 (1951).
13. J. L. Nicholson and J. Altman, *Brain Res.* **44**, 13 (1972).
14. T. R. Anderson and S. M. Schanberg, *Biochem. Pharmac.* **24**, 295 (1975).
15. S. M. Schanberg and T. R. Anderson, *Trans. Am. Soc. Neurochem.* **3**, 117 (1972).
16. K. Suzuki, *Life Sci.* **3**, 1227 (1964).
17. A. J. Horowitz and S. M. Schanberg, *Biochem. Pharmac.* **28**, 881 (1979).
18. H. J. Maccioni, A. Arce and R. Caputto, *Biochem J.* **125**, 1131 (1971).
19. G. W. Jourdian, L. Doan and S. Roseman, *J. biol. Chem.* **246**, 430 (1971).
20. J. Altman, in *Developmental Neurobiology* (Ed. W. A. Himwich), p. 197. Charles C. Thomas, Springfield, Ill. (1970).