Effects of neonatal thyroxine stimulation on adult open-field behavior and thyroid activity in rats

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Three experiments are reported in which small groups of rats, given either thyroxine (T₄) or triiodothyronine (T₃) in large doses on Day 3 or Day 4 after birth, were tested in an open-field situation as adults. Ambulation and rearing were significantly increased by neonatal thyroid hormone treatment in both male and female rats. The effects were more pronounced among males than among females and were reduced by adult handling in the female group. With advancing age, hormone-treated rats showed a progressively lower body weight than controls. No consistent effect on thyroid uptake of radioiodine was noticed. The results are discussed with respect to possible implications for the reported adult learning deficits in rats treated in a comparable manner.

Early postnatal thyroid hormone stimulation in rats has been reported to cause short-term developmental as well as persistent effects of several kinds. Significant reviews on the subject have been published by Eayrs (1964b, 1968), Schapiro (1971a, b) and Balázs and Cotterell (1972). A multitude of findings has been described, ranging from an accelerated development of the central nervous system during the preweaning period (Schapiro, 1968, 1971a, b) and a retarded body weight development (e.g., Eayrs & Holmes, 1964) to a slightly decreased thyroid activity in adulthood (Eayrs & Holmes, 1964; Schreiber & Kmentová, 1965/66; Bakke & Lawrence, 1966; Gellert, Bakke, & Lawrence, 1971). Behaviorally, rapid learning of an active avoidance response at 16-18 days of age (Schapiro & Norman, 1967) and an accelerated rate of development of swimming ability and other reflex patterns before weaning (Schapiro, Salas, & Vukovich, 1970; Davenport & Gonzalez, 1973) have been reported in rats treated with thyroid hormones neonatally. Also, thyroxine-stimulated animals have a high locomotion score in the open field at 9 (Schapiro, 1966) and at 30-40 days of age (Schapiro, 1968).

With regard to the adolescent and adult learning ability of rats treated with large doses of thyroid hormones in the early postnatal period, scattered reports indicate an impairment of learning in the Lashley III maze and a Y-shaped swimming maze at 35-45 days of age (Schapiro, 1968) and in the Hebb-Williams closed-field test at 100 days of age (Eayrs, 1964a). These findings have recently been corroborated and extended by Davenport and Gonzalez (1973). Whether or not adult hormone-treated rats differ from controls with regard to other behavioral parameters, such as exploratory behavior, does not seem to be known.

The aim of the present investigation is to elucidate the mechanisms possibly contributing to the reported impairment of adult learning ability in thyroid hormone

treated animals. Specifically, it will be assessed whether or not neonatal hormone treatment affects the adult behavior of rats in an open-field situation. The rationale behind this approach is that differences of exploratory behavior and/or emotionality between hormone-treated rats and controls, as assessed by the open-field test (Whimbey & Denenberg, 1967), might help to account for the reported impairment of adult learning ability. This approach is similar to that of Bronstein (1972), who attempted to explain the learning deficiency of juvenile rats as compared to adults in terms of a higher rate of exploratory reactions.

One major methodological difference from previous studies will be introduced in the present investigation. Most authors have employed multiple, small injections of thyroid hormones (Eayrs, 1964a; Davenport & Gonzalez, 1973). In view of the well-known effects of neonatal stimulation of varying kinds on adult behavior (Denenberg, 1964), multiple injections will be replaced by a single injection containing approximately the same total amount of hormone as that achieved by multiple injections in an attempt to reduce the nonspecific stimulation exerted by the repeated injections.

Body weight development will also be studied in the present investigation along with an estimation of the activity of the thyroid gland. These two factors might also be helpful in accounting for the impaired learning of food-rewarded tasks, e.g., the Lashley III (Schapiro, 1968), Hebb-Williams (Eayrs, 1964a), and symmetrical mazes (Davenport & Gonzalez, 1973), since hypothyroid animals eat significantly less than euthyroid controls (Fregly & Taylor, 1964) and may, therefore, be less motivated to work for food.

EXPERIMENT I

Method

Subjects and Housing Conditions. Seven nulliparous Wistar

females, approximately 100 days old, purchased from KE Møllegaard, Denmark, were made pregnant by housing them with one Wistar male each for 6 days. The females gave birth to viable litters consisting of from 7 to 14 pups ($N = 62$). The pups were randomly redistributed to the mothers within 24 h after birth, so that each mother received the same number of pups as that born. This was done in order to balance possible genetic and prenatal differences between biological litters. The offspring were weaned and sexed at 28 days of age and were then housed in one-sexed group cages, three to four animals in each, until Day 140, when individual housing was initiated. At weaning, 21 randomly chosen animals representing both sexes and treatment conditions approximately equally and, at Day 80, five female rats were deleted from this study and used for other purposes. This left 41 subjects from Day 28 to Day 80 and 36 subjects from Day 80 to Day 165. Subsequent to this age, only the animals used for open-field testing (see below) remained in the studied group. Food and water were available ad lib for the first 90 days, after which all animals received five food pellets of roughly equal size each day (Lab Chow 210, AB Anticimex, Sollentuna, Sweden). This equalled approximately 25 g per animal per day and exceeded the daily consumption of individual animals.

Injections. On Day 3 after birth, half of the animals in each litter were given an i.p. injection consisting of 50 μg l-thyroxine-sodium (T_4) (Nyegaard and Company, Oslo, Norway) dissolved in .25 ml isotonic saline (thyroxine group: T). The other half received an equivalent amount of saline (controls: C). For identification purposes, a small cut was made in the right ear of the T_4 -treated animals and in the left ear of the controls. Since the main interest of the present study is the effects of thyroid hormone proper, rather than the effects of nonspecific neonatal stimulation by injections and handling per se, no uninjected controls were used. The possibility of an independent action of the saline injection in a direction opposite to that of the hormone injection is indeed very slight, a fact that further reduces the necessity of an unstimulated control group.

Behavioral Testing. At approximately 140 days of age, four males and seven females were randomly chosen from each group (T and C). Ten days later, they were tested in an open-field test for 4 consecutive days, 4 min each day. The open field was an acrylate box (60 x 60 x 26 cm) with an open top, white sides, and a brown floor, divided into 16 equal squares (15 x 15 cm). The lines were painted on oil cloth, which was covered by an acrylate board. The box stood on the floor of a small windowless room, where an air conditioning system provided a constant background noise of approximately 51 dB (Bruel and Kjaer precision sound-level meter, Type 2203), measured in the center of the open field. Illumination consisted of light from a frosted 60-W bulb positioned 170 cm above the center of the field. The rat was gently placed in a corner square of the field closest to the position of the experimenter, who was unaware of the meaning of the ear-marking identification. Records were kept of the following behavior: *ambulation*—the number of squares traversed with all four feet, *rearing*—the number of times the rat rose on its hind feet, and *defecation*—the number of fecal boli deposited. After a rat had been tested, fecal boli and urine were removed and the floor was cleaned using tap water. The order of testing T and C animals and males and females was rotated over the 4 days. To ascertain the stability of possible differences between T and C groups in the open field at 150 days of age, a renewed testing of the same animals was carried out at 210 days. The procedure of the initial testing was maintained.

Body Weight. All animals available were weighed on the day of injection and subsequently at regular intervals until approximately 210 days of age.

Thyroid Uptake of Radioiodine. At the age of 240 days, the activity of the thyroid gland was assessed by a measurement of the 24-h uptake of radioiodine (I^{131}) of the gland. Each animal previously tested in the open field received a 1-ml i.m. injection

containing 40 μc of I^{131} . The animals were killed by ether 24 h later and a 3-cm portion of the trachea containing the larynx and thyroid gland was carefully dissected out and measured by a scintillation detector. Subsequent to this, the entire bodies deprived of the thyroid glands were measured in the same manner. The activity of the thyroid gland (TG), the body (B), and the TG/B ratio, expressed in arbitrary units, was obtained (cf. neck/thigh ratio in man: Howard, McAlister, & McEvedy, 1958). Within groups of rats of the same sex and feeding condition, there is a good correlation between the thyroid uptake of radioiodine and the release of labeled hormone (unpublished observations).

Results

Open-Field Behavior. The neonatal stimulation with thyroid hormones caused a significantly elevated rate of both ambulation and rearing in the open field for both sexes of rats. Defecation scores were unaffected. The differences between T and C groups were evident also at the testing 60 days subsequent to the initial test, with somewhat smaller effects appearing for the females.

Figure 1. Mean values of ambulation and rearing in the open field of 150-day-old rats treated neonatally with T_4 (●) and controls (○). Experiment I: initial testing. ♂ = males, ♀ = females.

Analysis of variance was carried out on the data according to Kirk (1968). Male and female groups were treated separately in this analysis. The design was a 2 by 4 split-plot factorial, with groups (T and C) as the between factor and days of testing (1, 2, 3, 4) as the within-groups factor.

Data from the initial testing are shown in Figure 1, where ambulation and rearing means over the 4 days are presented. Analysis of variance of ambulation scores indicates a significant effect of T_4 treatment for both males ($F = 16.37$, $df = 1/6$, $p < .05$) and females ($F = 4.98$, $df = 1/12$, $p < .05$). The identical pattern of effects is evident among males for rearing ($F = 6.77$, $df = 1/6$, $p < .05$), where the T group shows a higher rate than the C group. Although the difference between the female T and C groups is numerically equal to that between male T and C groups, it does not reach statistical significance. An inspection of the analysis indicates that this is due to the rather large variation of rearing scores in both female groups. The only significant factor in the analysis of defecation data is a general decrease over days for the total female group ($F = 3.27$, $df = 3/36$, $p < .05$).

Data from retesting in the open field 60 days later are shown in Figure 2. Analysis of variance of ambulation data yielded a significant thyroxine effect for the males ($F = 9.90$, $df = 1/6$, $p < .05$) but not for the females. Due to the rather large individual variations in both female groups, the striking numerical differences between T and C group means in Figure 2 did not reach statistical significance. A significant thyroxine effect is shown for rate of rearing among males ($F = 20.19$, $df = 1/6$, $p < .05$) but not among females. With regard to defecation, no difference appeared between T and C groups for either sex. The incidence of defecation was rather low for all groups during the initial as well as during the subsequent testing.

Body Weight. Separate *t* tests were carried out for each point of measurement. From Day 14 and onward to weaning, the T_4 -treated group weighed significantly less than the controls (*t* values ranging from 1.94 to 3.28, $df = 60$, $p < .05$). For the postweaning period, separate *t* tests were performed in the male ($df =$ from 12 to 6) and female groups ($df =$ from 25 to 12). The overall pattern emerging from this analysis shows that among the males the T group showed a significantly lower mean body weight than controls for all points of measurement (*t* values from 2.03 to 5.11, $p < .05$). In the female groups, the same pattern appeared, but the differences were smaller and reached significance only in a few instances, notably above 150 days of age (*t* values from 1.81 to 2.18, $p < .05$). Body weights recorded at 90 and 180 days of age are shown in Table 1.

Thyroid Uptake of Radioiodine. The mean uptake of radioiodine of the thyroid gland (TG), body (B), and the TG/B ratio were computed separately for each experimental group and sex. The results are presented in Table 2.

Figure 2. Mean values of ambulation and rearing in the open field of 210-day-old rats treated neonatally with T_4 (●) and controls (○). Experiment I: retesting. ♂ = males, ♀ = females.

The only significant difference was obtained between male T and C groups for absolute TG values, where the T group showed less thyroid activity than the controls. The TG/B ratios were not, however, significantly different.

Table 1
Body Weights at 90 and 180 Days of Age of Rats Treated With Thyroid Hormones Neonatally

	Approximate Ages			
	90 Days		180 Days	
	Males	Females	Males	Females
Experiment I				
T	196.3* (4)	174.3 (7)	311.8* (4)	207.3* (7)
C	225.3 (10)	183.1 (15)	332.3 (4)	219.9 (7)
Experiment II				
T	276.6* (7)	190.8 (6)	343.7* (7)	216.7* (6)
C	296.7 (6)	198.4 (5)	362.4 (6)	230.0 (4)
Experiment III				
T	266.0* (8)	179.4* (14)	324.0* (8)	212.5* (14)
C	291.8 (8)	188.5 (13)	359.6 (8)	222.3 (13)

Note—Mean weights in grams. Number of subjects in parentheses.

*Significantly different from controls, $p < .05$.

Table 2
Measurement of Body and Thyroid Uptake of Radioiodine

	Body Counts (B)		Thyroid Counts (TG)		TG/B Ratios		N	
	T	C	T	C	T	C	T	C
Experiment I								
M	1.33	1.34	1.48*	1.86	1.17	1.43	4	4
F	.99	1.21	2.74	2.79	2.99	2.69	7	7
Experiment II								
M	3.73	4.09	7.93	7.55	2.13	1.88	7	6
F	3.45	3.26	9.38*	7.58	2.72*	2.32	6	4
Experiment III								
M	3.10	3.16	4.29	4.84	1.40	1.53	8	8
F	2.17	2.09	3.43	3.02	1.59	1.46	14	13

Note—All values are expressed as arbitrary units of measurement. M = males, F = females.

*Significantly different from controls, $p < .05$.

EXPERIMENT II

In order to elucidate whether or not the differences of open-field behavior observed between T and C groups in Experiment I could be explained merely by differential reactions to handling and placing in the open field, another experiment was performed, employing a 10-day period of handling immediately prior to open-field testing. In this experiment tri-iodothyronine (T_3) was used instead of T_4 .

Method

Four nulliparous Wistar females, 100 days old, from the same breeder, were subjected to the same procedure as in Experiment I. Their litters consisted of five to nine pups ($N = 27$). An i.p. injection of 50 μ g tri-iodothyronine (T_3) dissolved in .1 ml saline was given to half of the animals in each litter on Day 4 after birth. Controls received saline only. One animal died immediately upon being injected. Identification, redistribution, weaning, housing, and weighing of the offspring were carried out as in Experiment I. At 140 days of age, five males and four females were randomly chosen from each group and housed in individual cages, like the remaining animals. Between the ages of 140 and 150 days, all animals were subjected to daily handling for 5 min each day. This chiefly consisted of picking up the animal in its home cage and moving it back and forth between this and another identical cage, and was carried out by a technician unaware of the purpose of the procedure and the identification of the animals. After the handling period, open-field testing was carried out as in Experiment I. Body weights were recorded in all animals (initial $N = 26$ reduced by deaths to $N = 23$) every second week from the day of injection until 210 days of age. Thyroid uptake of radioiodine was estimated in all animals, as in Experiment I, at 210 days of age. Prior to the assessment of thyroid activity, the animals were kept on a iodine-free diet for 5 days (Astra-Ewos, Södertälje, Sweden).

Results

Open-Field Behavior. Among the males, neonatal treatment with tri-iodothyronine (T_3) caused an increased ambulation and rearing in the open field in spite of the 10-day intervening handling period. No differences appeared among the females.

Analysis of variance indicates that the T_3 treatment increased the rate of ambulation ($F = 68.42$, $df = 1/8$,

Figure 3. Mean values of ambulation and rearing in the open field of 150-day-old rats treated neonatally with T_3 (●) and controls (○) after 10 days of prior handling. Experiment II. ♂ = males, ♀ = females.

$p < .05$) and rearing ($F = 10.96$, $df = 1/8$, $p < .05$) in the male group. This is illustrated in Figure 3. No difference appeared between the female T and C groups. The total female group changed its rate of ambulation significantly over days ($F = 4.58$, $df = 3/18$, $p < .05$). No effect of the experimental treatment was observed among the defecation scores.

Body Weight. The same trends as were evident in Experiment I showed up in the present results. T_3 -treated animals had a lower mean body weight than controls at all points of measurement for both sexes, but the differences were smaller than in the previous experiment and reached statistical significance only after 120 days of age in both sex groups [t tests from 1.67 to 3.52, df (males) = 11, df (females) = 9, $p < .05$]. Body weights recorded at 90 and 180 days are given in Table 1.

Thyroid Uptake of Radioiodine. As seen from Table 2, the female T group showed a higher activity of the thyroid gland than controls, expressed both as absolute TG values and as TG/B ratios.

Figure 4. Mean values of ambulation and rearing in the open field of 140-day-old rats treated neonatally with T_3 (●) and controls (○). Experiment III. ♂ = males, ♀ = females.

EXPERIMENT III

Since T_4 was used in Experiment I and T_3 in Experiment II, conclusions with regard to the possible differential effects of the two thyroid hormones on adult open-field behavior are not entirely clear. For this reason, an experiment utilizing a large dose of T_3 with no explicit handling period prior to open-field testing was performed.

Method

Five nulliparous Wistar females, 100 days old, were made pregnant by the same procedure as in Experiments I and II. Their litters were made up of from 7 to 12 pups ($N = 48$). On Day 3 after birth, an i.p. injection consisting of 200 μ g triiodothyronine (T_3) in .25 ml saline was given to half of the animals in each litter. Controls received saline only. The identical procedure as in previous experiments was used for identification, redistribution, weaning, housing, etc. At the age of 130 days, 8 males and 12 females were randomly chosen from each group (T and C) and were, like the remaining animals, housed in individual cages. Open-field testing was begun when

the animals were approximately 140 days old. Body weights of the entire group (initial $N = 48$ reduced by three preweaning deaths and two deaths on Day 75 to $N = 43$) were recorded at intervals of approximately 10 days until 180 days of age. Thyroid uptake of radioiodine was assessed in all 43 animals by the same procedure as in Experiment II at the age of 195 days.

Results

Open-Field Testing. The same overall results as in Experiment I were obtained, with the exception that neonatal hormone treatment did not increase rearing scores for males.

Analysis of variance indicates that there is a significantly higher rate of ambulation in the T groups, both among males ($F = 4.60$, $df = 1/14$, $p < .05$) and females ($F = 4.71$, $df = 1/22$, $p < .05$). This is also clearly seen in Figure 4. Rearing means are illustrated in Figure 4 also. Analysis of variance yielded a significant augmenting effect of thyroid hormone among the females ($F = 8.43$, $df = 1/22$, $p < .05$) but not among the males. The total groups decreased their rate of rearing significantly over days (males: $F = 3.73$, $df = 3/42$, $p < .05$; females: $F = 10.78$, $df = 3/66$, $p < .05$). A decrease of defecation over days was significant for the total female group ($F = 4.79$, $df = 3/66$, $p < .05$).

Body Weight. The identical pattern of body weight development as in Experiment I appeared. Separate t tests for the 15 points of measurement indicated that T_3 -stimulated males weighed significantly less than controls beginning at 60 days and onward (t tests from 1.87 to 2.53, $df =$ from 16 to 14, $p < .05$). The difference between the female T and C groups was smaller, but reached statistical significance after about 130 days and onward (t values from 1.91 to 2.70, $df = 25$, $p < .05$). Body weights at 90 and at 180 days are given in Table 1.

Thyroid Uptake of Radioiodine. As seen from Table 2, no significant difference was observed between any of the groups with regard to this variable.

DISCUSSION

Results from three experiments reported here indicate that neonatal treatment with a single heavy dose of either T_3 or T_4 produces rats which are far more active than untreated animals in an open-field test, performed as late as 140-200 days after the injection. That the action of hormone treatment is indeed specific to the exploratory tendencies of the animals and does not involve an action on their emotionality (Whimbe & Denenberg, 1967) is suggested by the lack of effects on defecation.

Although rather small groups of rats were used, the highly consistent results in separate experiments strongly support the generalization of this conclusion to other groups of animals. The random procedure used to distribute animals to the mothers post partum admittedly entails a certain risk that several animals from the same biological litter may have ended up in the same treatment group. This may have created a correlation between certain scores, thereby possibly leading to a spuriously low estimate of error variance and an inflated F value. However, the chance that this would happen in three separate experi-

ments is small, much smaller than the probability of getting offspring representing the extreme tails of the activity distribution in the same group, which would have lead to a spuriously high error variance.

The conclusion by Davenport and Gonzalez (1973), with regard to a temporary superiority of locomotor ability in neonatally T_4 -treated rats during Weeks 2 and 3 after birth is not substantiated by our findings. In place of an advanced maturation of locomotor systems (reflected in a facilitation of swimming and other reflex patterns) only during the second and third weeks of life (*ibid.*), the T_3 - and T_4 -treated animals seem to be permanently more active than their controls. The effects are reflected in ambulation as well as rearing scores and are somewhat more stable and replicable for males than for females. The augmenting action of neonatal T_3 or T_4 on the exploratory behavior of female rats was reduced by handling prior to open-field testing (Experiment II), and also less marked when the animals were subjected to retesting in the open field (Experiment I). If prior handling and retesting are considered as manipulations reducing the degree of novelty of the open-field procedure, it can be concluded that such a reduction tends to efface the differences between female experimental and control animals with regard to exploration.

Lát and Gollová-Hémon (1969) have previously reported that neonatal thyroxine (T_4) injections caused increased rates of rearing in rats at 40 days of age and also a slower rate of behavioral habituation to novel settings up to 200 days. Our findings replicate and extend these data and also corroborate the lack of effects on "lability" (emotionality) as measured by the defecation variable, reported by these authors (*ibid.*). Other reports on activity/exploration in postnatally thyroxine-treated animals have indicated a higher locomotion score for rats in the open field up to 35-40 days of age (Schapiro, 1966, 1968) and for hamsters at 20 but not at 30 and 65 days of age (Darling, 1966).

In view of the well-known effects of varying kinds of infantile stimulation on adult open-field behavior in rodents (Denenberg, 1964; Goldman, 1969), the possibility should be considered that the injection of thyroid hormones has acted as a nonspecific stressor in the experiments reported here (cf. Swanson & McKeag, 1969). Increased rates of ambulation have been reported for adult rats subjected to infantile handling and, generally, their rate of defecation is decreased to a substantial degree (Denenberg, 1964; Goldman, 1969). The specific increases of ambulation and rearing and the lack of effects on defecation in the present results make it unlikely that the data could be explained entirely in terms of nonspecific infantile stress experience. Furthermore, it should be noted that, whereas neonatal handling generally results in a higher body weight as compared to controls (but see Denenberg, 1964), neonatal thyroxine injections induce the reverse effects, as mentioned above.

The implications of the present findings for the understanding of the reported adult learning deficits in neonatally thyroid hormone treated animals seem to be as follows. The high rates of exploratory behavior in the T groups might put these animals at a disadvantage in certain learning situations. In the tests used by previous researchers, the T groups have been reported to commit more errors in the Lashley III maze and a Y-shaped water maze (Schapiro, 1968), the Hebb-Williams closed-field test (Eayrs, 1964a), and the symmetrical maze (Davenport & Gonzalez, 1973), and to show a deficient inhibition of responding in the passive avoidance test (Davenport & Gonzalez, 1973). Active avoidance conditioning has been reported not to differentiate between T and C groups (*ibid.*). In terms of the number of trials required for attainment of the criterion of learning, the first four above-mentioned tests seem to be of a fairly high degree of complexity. It might be that the difficulty of the learning test, rather than the developmental age at which the animals are tested (Schapiro, 1968; Davenport & Gonzalez, 1973) is a determining factor with regard to the performance

of T and C groups. Following this line of reasoning, T groups might even show superior performance in certain "simple" tasks such as one-way active avoidance (Schapiro, 1968). Other, somewhat more difficult, tests might fail to discriminate between T and C groups, which was the case for the two-way active avoidance in the study by Davenport and Gonzalez (1973). At the present time, a study is in progress at our laboratory, where it will be investigated whether or not T groups show superior performance to C groups on "simple" tests of learning.

The single heavy injection of thyroid hormones employed in the present study, as compared to the relatively small multiple injections in the studies by Eayrs (1964a) and Davenport and Gonzalez (1973) is a factor to be taken into consideration when comparing results from these different laboratories. However, several indications point to the conclusion of a relative comparability of the two procedures, at least with respect to effects on adult learning. First, the total amount of hormone given via a number of small injections in previous studies (Eayrs, 1964a; Davenport & Gonzalez, 1973) is in the same dose range as that given in the present study. Second, with respect to adult learning in neonatally hormone treated animals, we have recently found comparable effects to those reported by the previous workers (*ibid.*) in the Hebb-Williams maze and in reversal learning in a test of spatial discrimination (water-filled T-maze) (to be published).

In the three experiments reported here, there is evidence that the neonatal treatment with thyroid hormones has caused a permanent decrement in the growth rate of the treated animals. This is particularly true for males and especially so for the ones treated with T_4 . The weight differences, furthermore, seem to increase with age. These findings agree well with earlier reports (Eayrs & Holmes, 1964; Schreiber & Kmentová, 1965/66; Bakke & Lawrence, 1966; Best & Duncan, 1969; Lát & Gollová-Hémon, 1969; Gellert, Bakke, & Lawrence, 1971; Davenport & Gonzalez, 1973). The body weight reduction has been proposed to result from the previously mentioned hypothyroid state in neonatally thyroxine treated animals (Eayrs & Holmes, 1964), but this suggestion has been rendered relatively unlikely in view of the failure of replacement treatment with T_4 to appreciably change body weight gain (Best & Duncan, 1969). The suggestion that the effects on body weight might be mediated via a reduced production of growth hormone has not received support (Eayrs & Holmes, 1964; de Raveglia, Gómez, & Ghittoni, 1973). As proposed by Best and Duncan (1969), an increased maturation speed of the neuroendocrine system controlling slowing and cessation of growth seems a more tenable hypothesis as of today.

Our data from the thyroid uptake of I^{131} do not support the previously found relation between neonatal thyroid hormone treatment and a slight degree of adult hypothyroidism (Schreiber & Kmentová, 1965/66; Bakke & Lawrence, 1966; Best & Duncan, 1969). However, we have recently observed (unpublished observations) that an i.m. dose of T_4 , corresponding to the daily endogenous secretion in adult rats, will activate the deiodinating enzymes significantly for at least a month after the injection. If this phenomenon holds for newborn rats as well, the neonatal injection will yield an increased rate of peripheral utilization of hormone with a corresponding increase of the demand of hormonal secretion from the thyroid in these animals. In this way, a false impression of an increased thyroid activity may have been created in our T groups and a true fall may have been obscured.

The behavioral indicators (ambulation, rearing) do not evidence that the animals are hypothyroid, since the traditional view has been that hypothyroidism is accompanied by low motor activity (Richter, 1933; Hall & Lindsay, 1938; Brody, 1941, 1942; Stern, 1970) as measured by the revolving-drum technique. Other reports (Feuer & Broadhurst, 1962) show that this low motor activity is not specific to the running-wheel method. The traditional view has recently been questioned by

Dupont, Endröczy, and Fortier (1971), who found that surgical thyroidectomy during the adult period caused high rates of locomotion and rearing in a two-compartment test. Thus, we might conclude that a single heavy dose of hormone does not produce adult hypothyroidism, whereas small multiple injections do (Schreiber & Kmentová, 1965/66; Bakke & Lawrence, 1966). This is supported by the observations of Bakke, Lawrence, and Robinson (1972), who found single injections of large amounts of T_4 ineffective in producing the "neo- T_4 " syndrome (secondary mild hypothyroidism). An alternative interpretation would be that the hypothyroidism, which may have been obscured in our animals, is accompanied by a high frequency of locomotor exploration. We thereby add further doubts to the previously held view that hypothyroidism causes motor inactivity. Still, there remains the possibility that the neonatal treatment causes another type of disturbance of the pituitary-thyroid axis than adult interventions, or that we have affected a so far unknown third factor permitting both hypothyroidism and high motor activity. Neural as well as endocrine systems will have to be considered in future research on this matter.

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