THE EFFECTS OF NEONATAL TREATMENT WITH METHYLTHIOURACIL AND TRIIODOTHYRONINE ON PHYSICAL AND BEHAVIOURAL DEVELOPMENT

PROEFSCHRIFT

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INTRODUCTION

The important role of hormones in regulating physiological processes in man and animals has been well established by many workers in endocrinology. The site and mode of action of hormones at the cellular level, however, is not yet clear. This is also true for the thyroid hormones L-thyroxine and L-triiodothyronine. The thyroid hormones accelerate many biological processes; examples are their calorigenic and protein anabolic action. It is not yet certain whether the thyroid hormones have different distinct actions or that their different effects are manifestations of one single action at the cellular level. During the last decade evidence has been put forward for the view that thyroid hormones act on the mitochondria and on transcriptional processes involved in gene expression (Tata et al., 1963; Tapley, 1964; Tata, 1964, 1965, 1966, 1968, 1971).

The metabolic effects of thyroid hormones, particularly their promoting effect on protein synthesis, give them an important role in growth and other aspects of development. Hypothyroidism, due to congenital dysfunction of the thyroid gland or to endemic cretinism, is the best known thyroid disorder in the human, with dwarfism and severe mental retardation as the most striking symptoms (reviews by Smith et al., 1957; Ramalingaswami, 1964; Shellabarger, 1964; Andersen, 1969; Koenig, 1969; van Rhijn, 1969). In experiments with animals, predominantly rats, short-term and long-term effects have been observed of temporary and lasting thyroid hormone deficiency or excess, beginning shortly after birth or at some time later in life, on physical development as well as on the maturation and development of regulatory mechanisms, nervous system and behaviour. A schematic review of the relevant literature on these effects is given below in table 1.

An important aspect of the hormonal influences on development is the existence of a developmental period during which the presence or absence of a hormone is decisive for the adult organization of the individual. Such a "critical period" exists in the rat during the first postnatal weeks, when its endocrine regulation system is not yet fully mature and functionally competent. Some endocrine and neural responses are present at a very rudimentary level, if present

at all. Experimental alterations in hormone levels during this neonatal period have been shown to produce profound changes in the further development of the animal, especially of the brain (for a concise review see Levine & Mullins, 1966). The rat brain goes through a short period of rapid development until the third week after birth; this has been established by determination of the ratios of DNA, RNA and protein in the brain at different ages during this period (Winick & Noble, 1965; Oja, 1966, 1967). The important influence of thyroid hormones on metabolic processes will make the brain particularly vulnerable for deviations from the euthyroid state during the first postnatal weeks, as can be deduced from the results of many recent studies (table 1).

Many of these effects of early deviations from the euthyroid state may also be obtained by early food deprivation (reviews by Altman et al., 1970, 1971; De Natris, 1971). On these grounds authors such as Tusques (1956) and Myant (1966) express their doubts about the existence of any specific effect of thyroid hormones on development; deviations from the euthyroid state would affect the availability of nutrients at the cellular level and exert their effects in that way. The last few years, however, investigators such as Hamburgh (1966, 1968) have collected evidence that the timing of cell differentiation might be such a specific effect of thyroid hormones, wrong timing due to abnormal thyroid hormone levels causing distortions of the chemical and histological composition of tissues and organs, because cell formation and cell differentiation are mutually exclusive events.

In order to clarify the ways along which the thyroid exerts its influence on development, it seems necessary to distinguish between lasting and temporary effects of short periods of hypo- and hyperthyroidism during early infancy, and to study quantitative developmental parameters such as body growth and behavioural scores as well as qualitative ones such as maturation of structures and functions and their interrelationships. It also seems useful to look for possible differences between a short period of severe hyperthyroidism and a longer period of mild hyperthyroidism in order to collect data on the relative importance of dosage versus duration. Triiodothyronine, with its short biological half-life as compared with thyroxine, will be likely to give the best results in tracing such differences. In the present study an attempt is made to contribute to the approach indicated above by investigating the relations between body growth and skeletal maturation as they are affected by treatment with thyroid hormone or antithyroid drugs during a restricted neonatal period. By collecting longitudinal data on these phenomena it becomes possible to analyse the effects of the treatments on developmental patterns. Little is known of such patterns, because the majority of earlier studies has dealt with animals that had been hypothyroid throughout the experimental period or had been measured just a few times and usually at rather early ages.

The second aim of the present study is to investigate whether lasting ef-

fects occur of early hypo- and hyperthyroidism on adult behaviour. As thyroidectomy is known to produce a decrease in locomotor activity in rats (table 1), the effects of the neonatal treatments on this activity were measured at 1 and 3 months after birth. In view of the persistence of mental retardation in cretins mentioned above, learning ability was tested at ages between 4 and 6 months. The emergence of some indices of neural maturation was studied in order to establish possible changes in their timing.

The neonatal treatments given in the present experiments may have an organizational effect on the adult endocrine system, particularly on the brain-pituitary-thyroid axis, like neonatal presence of androgen has on the adult pattern of gonadotropin secretion (Neumann et al., 1970). In order to establish if the effects on physical and behavioural development are indeed caused by the abnormal thyroid hormone levels in infancy and not by persistent gross abnormalities of the endocrine system, possible effects of the treatments on the endocrine system were looked for. This was done by determining the effect of a low environmental temperature on serum thyroxine levels and by collecting autopsy data not only on brain weight, but also on the weight of thyroid, adrenal, testes and seminal vesicles.

TABLE 1. Survey of litterature on the effects of thyroid hormones on development. Treatments are indicated as follows: TX = thyroidectomy; TU = thiouracil (anti-thyroid drug); T3 = triiodothyronine (thyroid hormone); T4 = thyroxine (thyroid hormone); TF = thyroid feeding. Unless stated otherwise the studies referred to in this table concern the rat.

Nature and time of treatment	Nature of effect	Authors
	BODY GROWTH	
neonatal TX	persistent dwarfism	Ray et al., 1950 Salmon, 1936,1938 Scow & Simpson, 1945 Scow et al., 1949
	growth reinstated by T4 or growth hormone	Ray et al., 1950 Scow et al., 1949
TU from day 1	lower body wt. on days 15 and 24	Eayrs & Taylor, 1951
TX after weaning	no further growth, normal growth with T4 replacement	Evans et al., 1939 Eartly & Leblond, 1954
acetyl-T4 from day 1	lower body wt. on days 15 and 30	Hoskins, 1927
T3 3 μg/days 1-24 or 30 μg days 2-4	great reduction in body wt. on days 35 and 60	Eayrs & Holmes, 1962,1964
T3 30 μg days 14-20	normal body wt. on day 60	Eyars & Holmes, 1962,1964
T3 or T4 single dose neonatally	growth retarded	Swanson & Van der Werff ten Bosch, 1965
	persistent reduction in body wt. with min. dose of 15 μg/100 g body wt.	Khamsi & Eayrs, 1966
T4 daily after weaning	acceleration of growth: same ultimate body wt. reached earlier in mice	Koger et al., 1942

	SKELETAL MATURATION	
neonatal TX	severely retarded up to 1-2 months of age	Salmon, 1936,1938 Scow & Simpson, 1945 Scow et al., 1949 Ray et al., 1950 Hamburgh & Lynn, 1964 Hamburgh, 1968
neonatal T3 or T4	advanced	Hoskins, 1927 Swanson & Van der Werff ten Bosch, 1965
	THERMOREGULATION	
TX before day 10	impaired on day 20	
TX after day 10	not affected on day 20	Hamburgh, 1968
	ENDOCRINE SYSTEM	
neonatal TX or TU	thyroid persistently enlarged; elevated TSH content in hypothalamus and pituitary; slightly diminished serum concentration of thyroid hormones; delayed puberty, lengthened oestrus cycles	Bakke et al., 1970
	gonads very small, opening of vagina delayed until day 100	Scow & Simpson, 1945
TX at or TU from 3 weeks	seminal vesicles + coagulation glands not affected on day 40; response to pregnant mare serum enhanced: increased weight of testes and seminal vesicles	Meites & Chandrashaker, 1949
	testes wt. lower on day 100; prevented by T3, not by TSH	Hara, 1963

TABLE 1, continued

TX after weaning	adrenals very small	Evans et al., 1939
	no normal ovarian cycles	Evans et al., 1939 Mann, 1945 Stern, 1970
	blood contains more TSH, but less growth hormone atrophy of pituitary target organs	Contopoulos et al., 1958
neonatal T3 or T4	smaller thyroid and pituitary	Eayrs & Holmes, 1964
	thyroid disproportionally smaller, impaired goitre response to thiouracil, delayed puberty	Bakke & Lawrence, 1966,1969
	no effect on thyroid activity	Swanson & Van der Werff ten Bosch, 1965
TF from about 3 weeks	lower testes wt. when mature; sexual maturity delayed	DaCosta & Carlson, 1933
	no effect on seminal vesicles at day 40; response to pregnant mare serum decreased	Meites & Chandrashaker, 1949
T4 adult	greater reproductive capacity in females (more corpora lutea and implantations, greater litter size)	Schultze & Noonan, 1970
	CHEMICAL AND HISTOLOGICAL BRAIN DEVELOPMENT	
neonatal TX or TU	brain wt. reduced after weaning by reduced cell size, cell formation unaffected or increased (protein/DNA ratio decreased)	Geel & Timiras, 1967,1970,1971 Pasquini et al., 1967 Balász et al., 1971
	protein synthesis reduced (incorporation of labelled amino acids)	Geel et al., 1967 Balász et al., 1971

	respiration in immature rat brain decreased; restored by T4 or growth hormone	Gomez, 1971
	myelination and myelin lipid deposition reduced	Balász et al., 1969,1971 Walravens & Chase, 1969
	activity of mitochondrial enzymes decreased	Hamburgh & Flexner, 1957 Argiz et al., 1967 Pasquini et al., 1967 Gomez, 1971
	differentiation of cerebral neurons impaired (less outgrowth of neurons, greater cellular density)	Eayrs, 1959,1960,1968 Eayrs & Lishman, 1955 Eayrs & Horn, 1955 Horn, 1955
	retarded differentiation of neurons and neuropil, delayed maturation of ribosomes, decrease in number of axodendritic and axosomatic synapses in cerebral cortex	Moskovkin & Mitskevitch, 1969
	retardation in many aspects of histological development of the cerebellum	Legrand, 1963,1967,1971 Legrand, Kriegel & Jost, 1961 Legrand & Bout, 1970 Clos & Legrand, 1970 Rebière & Legrand, 1970 Hamburgh, 1968 Hamburgh et al., 1971
neonatal T3	brain wt. reduced after weaning by reduced cell formation; no effect on cell size and protein synthesis	Balász et al., 1971
T4 in vivo and in vitro	amino acid incorporation increased in immature rat brain	Gelber et al., 1964 Sokoloff, 1967,1970
	myelination enhanced in vitro	Hamburgh, 1966

TABLE 1, continued

	FUNCTIONAL MATURATION OF THE BRAIN	
neonatal TX or TU	emergence of reflexes delayed	Eayrs & Taylor, 1951 Eayrs & Lishman, 1955
	elevated brain excitability (lowered electroshock seizure threshold) and increased capacity to sustain maximal activity (maximal electroshock seizure pattern with shorter flexion and longer extension)	Meisami et al., 1970
neonatal T3 or T4	emergence of reflexes advanced	Eayrs, 1964 Khamsi & Eayrs, 1966 Schapiro, 1968
	advanced maturation of electrocortical responses to different sensori stimuli, swimming ability and different behavioural responses	Schapiro, 1966,1968 Schapiro & Norman, 1967 Schapiro et al., 1970 Salas & Schapiro, 1970
	LOCOMOTOR ACTIVITY	
neonatal TX	"activity higher than expected"	Scow & Simpson, 1945
adult TX or TU	activity decreases	Richter, 1933 Hall & Lindsay, 1938 Mann, 1945
neonatal T4	elevated (open field) 6th week	Schapiro, 1968
adult TF	no effect	Mann, 1941
	with large amounts decrease of activity	Richter, 1933

LEARNING ABILITY

neonatal TX or TU	lower performance on three table test and elevated T-maze on day 60, repaired by medication from day 25 or earlier	Eayrs & Lishman, 1955
	lower performance on closed field test (Hebb-Williams maze) on day 100, repaired by medication from day 10-24	Eayrs, 1961
	lower performance on water escape test	Hamburgh et al., 1964
neonatal or adult TX	lower performance in conditioned avoidance learning, in both groups repaired by medication from as late as 5 months after birth	Eayrs & Levine, 1963
neonatal T3 or T4	lower performance on two maze-learning tasks at the age of 5-6 weeks	Schapiro, 1968
	lower performance on closed field test on day 100	Eayrs, 1964
	no effect on performance in water escape test	Hamburgh et al., 1964
T3 day 14-20	normal performance on closed field test	Eayrs, 1964

GENERAL METHODS

Animals and treatments

All animals used in this study were male F_1 hybrids of two inbred Wistar strains. They were born from R (Amsterdam) mothers and U (Amsterdam) fathers. There were two reasons for using these hybrids. In the first place the well known heterosis-effect gives hybrids a sturdier physical constitution than inbreds, so we could expect a smaller number of deaths following treatment. Indeed, there were no deaths before weaning at all. In the second place hybrids show less biological variance due to environmental conditions than inbreds, especially in such behavioural measures as used in this study; this has been shown in mice by Barnett & Smart (1970). Altogether this choice made it possible to work with relatively small numbers of animals.

Newborn litters containing at least eight males were reduced in size so that eight male pups per litter remained on day 1 (day 1 is the day when litters were born). Litters with fewer than eight males were sometimes employed, but then females were used to obtain a littersize of eight for the duration of the suckling period; on day 22 such females were eliminated. Each litter was randomly assigned to one of three treatment groups: A, B and C. Half the number of males in the litter was randomly chosen to receive a daily injection with:

A. MTU (methylthiouracil) in Na-alginate solution; 2 mg on days 2-8 and 4 mg on days 9-15.
B. T3(L-triiodothyronine) in 50% propyleneglycol; 0,5 μg on days 2-15.
C. T3 in 50% propyleneglycol; 40 to 50 μg on days 2, 3 and 4.

The other males in the litter received equal volumes of the appropriate solvent only; they served as controls.

Two litters of each treatment group were used for growth studies, involving weekly weighing, measuring and X-raying, as well as for the study of the emergence of reflexes. Six more litters of each group were used for the behavioural studies and the collection of data on endocrine organs.

All animals were weaned on day 22 and fed ad lib. with standard AM 2 pellets (Hope Farms Ltd.). They were housed 3 or 4 per cage, controls and ex-

perimental animals of each litter being separated at weaning, except for the animals used for the recording of locomotor activity, which were kept in littermate pairs. The room temperature averaged 22°C; the lights were on from 07.00 h till 21.00 h and the lights were off from 21.00 h till 07.00 h.

Statistical method

All data were subjected to the Wilcoxon two-sample test as described by Wabeke & Van Eeden (1955). This test is identical to the commonly used Mann-Whitney U-test. The reason for using the former was that this gives exact p-values, which the latter does not.

In all tables on the results of this study these exact p-values are given when between 0,01 and 0,1. Values greater than 0,1 are referred to as "NS" (not significant), values smaller than 0,01 as "S" (significant).

PHYSICAL DEVELOPMENT

Material and methods

Body growth and skeletal maturation

From the animals of two litters of each treatment group weekly measurements were taken from day 2 onwards. Length of head + trunk was measured in mm with a "Western Reserve Measuring Board" as described by Acheson et al., (1959) and will be referred to as body length. The animals were weighed on a Berkel balance to the nearest gram. From the age of 3 weeks onwards this was done under ether anaesthesia, which was necessary for the X-ray procedure described below. In tables 2, 3 and 4 the results of this measurements for the three treatment groups are given as length and weight gain since day 2 by subtracting the initial lengths and weights.

In order to smooth out the irregularities that are unavoidable in weekly measurements from relatively small numbers of animals, the growth velocity curves (fig.2 and 4) were drawn from the "running average", which means that each point represents the group-mean of the average weekly length or weight gains over periods of three successive weeks, the first point for weeks 1, 2 and 3, the second for weeks 2, 3 and 4, etc.

The differences in length and weight between the three experimental groups and their controls are represented by plotting the control values on the zero line (fig.3 and 5).

Of the same animals weekly X-rays were taken from three weeks after birth onwards. The animals were anaesthetized with ether and fixed on a Kodak No-Screen X-ray film with sellotape. This was done with the forelegs stretched, the palms upward and downward respectively, the left hindleg stretched and the right one bent, in order to obtain two different views of each ossification centre on the X-ray. Subsequently the animals were X-rayed with a Philips Praktix during one second from a distance of approximately 1 m. From these X-ray graphs scores for skeletal maturity were determined, using the rating method of Hughes & Tanner (1970). This method distinguishes a number of maturity

stages for each bone, as illustrated for the radius in fig.1. In the present study one single stage was assigned for each type of bone, when the stage was present at both sides. The bones used were (maximal score between brackets): humerus (14), radius (14), ulna (15), metacarpals (13), proximal phalanges (11), femur (15), tibia (15), calcaneum (9), metatarsals (13), proximal phalanges (11), and caudal vertebrae 7 +8 +9 (3 x 13). The maximal total score an animal could reach in this way was 169. The total scores are given in table 5, the differences are plotted in fig.6 in the same way as for length and weight in fig.3 and 5.

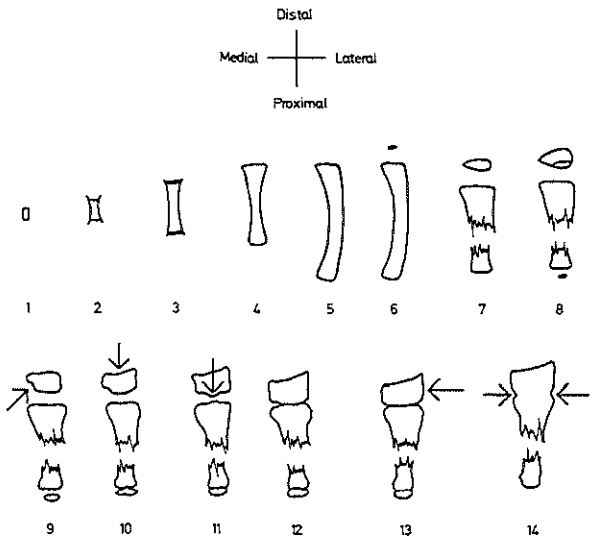


Fig.1. The 15 maturity stages of the radius (after Hughes & Tanner, 1970).

From each score of the experimental groups the skeletal age was calculated by interpolation from the corresponding control data. These skeletal age data were used to plot body length against skeletal age in fig.7.

Thyroxine levels

Blood serum thyroxine levels were studied in representative animals of the various treatment groups at the age of about 6 to 7 months.

Blood was collected before and after a one-week stay in a cold room at 5°C. About 1,5 ml blood was taken from the orbit, put in the cold room for several hours, then serum was extracted with ethanol. Total serum thyroxine concentrations were determined by staff members of Dr. G. Hennemann and

Ir. R. Docter at the Department of Internal Medicine III of the Medical Faculty Rotterdam. This was done by means of the Tetrasorb-125 T4 Diagnostic Kit, manufactured by Abbott Laboratories (Chicago, Illinois 60064, U.S.A.), a standardized protein-binding assay.

Autopsy data

A few weeks after the determination of serum thyroxine levels the animals were killed in chloroform in order to perform autopsy.

After killing the animals, body weight and length were measured in the same way as described above. After removal and weighing of the thyroid gland the rats were decapitated. The skull was carefully opened, the brain cut off at the foramen magnum, removed after cutting the optic nerves and weighed. Finally both adrenals and testes were removed, cleaned and weighed, and the seminal vesicles together with the coagulation glands were removed, opened, emptied and weighed. All organs were weighed on a Mettler balance to the nearest mg.

Results and Discussion

Body growth and skeletal maturation

The effects of treatment with thiouracil during two weeks in the neonatal period on body growth are shown in table 2 and figures 2A, 3, 4A and 5. A marked growth retardation was present, beginning in the second week and lasting for about four weeks for length and some time longer for weight. After this, however, the MTU-treated animals showed greater weekly length and

TABLE 2. Mean length and weight gain ± SEM since day 2 in rats of treatment group A (MTU days 2-15, 6 experimental and 6 control animals).

AGE WEEKS	LENGTH GAIN			WEIGHT GAIN		
	Exp.	Contr.	p	Exp.	Contr.	p
1	17,2 ± 1,1	15,2 ± 1,3	NS	8,2 ± 0,9	6,7 ± 1,3	NS
2	29,7 ± 0,7	36,2 ± 2,6	0,047	18,8 ± 1,8	21,3 ± 3,2	NS
3	46,3 ± 1,4	56,3 ± 4,0	0,066	26,5 ± 2,5	34,3 ± 3,8	0,090
4	56,2 ± 3,3	72,3 ± 4,9	0,017	30,5 ± 3,9	51,2 ± 6,3	S
5	80,3 ± 4,7	99,8 ± 5,3	0,013	55,7 ± 6,5	88,8 ± 9,0	0,013
6	97,2 ± 3,9	118,0 ± 4,4	S	91,2 ± 8,2	128,2 ± 11,1	0,021
7	116,0 ± 3,4	132,7 ± 4,6	0,017	126,7 ± 9,4	168,3 ± 13,3	0,013
8	133,0 ± 3,2	147,5 ± 3,7	0,021	163,2 ± 10,9	208,2 ± 15,3	0,021
9	139,2 ± 3,4	156,0 ± 3,5	S	195,7 ± 12,7	228,5 ± 15,6	NS
10	150,0 ± 4,3	158,0 ± 3,4	0,090	217,5 ± 13,2	250,2 ± 16,8	0,066
11	153,2 ± 3,7	161,8 ± 4,2	0,090	233,7 ± 14,3	262,5 ± 17,1	NS
14	164,8 ± 3,4	170,8 ± 3,1	NS	278,8 ± 15,8	301,5 ± 18,1	NS

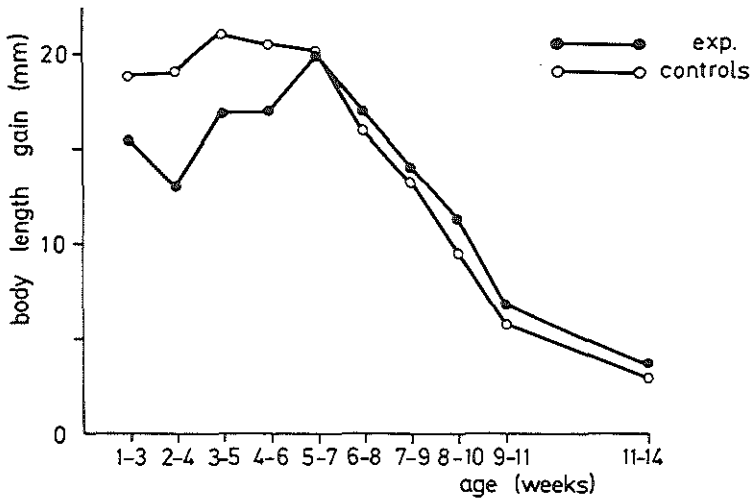
weight increments than their controls, ultimately reaching perfectly normal body size, but at a later than normal age. The autopsy data, taken from other individuals, show that at the age of 232 days there are no differences in body size between experimental and control animals (table 7). The growth velocity curve for weight (fig.4A) is almost similar in shape to that of the control animals, but is moved along the time scale.

The initial growth retardation is by no means an unexpected effect, as several earlier reports have shown that neonatal thyroidectomy results in persistent dwarfism (table 1). This does not imply that thyroid hormones have a direct growth promoting effect. Thyroid hormone medication will restore normal growth rates in thyroidectomized rats, but fails to do so in rats that are also hypophysectomized, whereas STH (growth hormone or somatotropin) will at least partly restore normal growth in thyroidectomized rats (Evans et al., 1939; Scow et al., 1949; Asling et al., 1954; Eartly & Leblond, 1954; Goodall & Gavin, 1966). These facts have led to the generally adopted view that the effects of hypothyroidism on growth are mediated by the pituitary. This view received support from the findings of Contopoulos et al. (1958), who found that in male rats thyroidectomized at the age of 45 days and killed 21 or 56 days later, the plasma seemed to contain more TSH and less STH than that of unoperated controls.

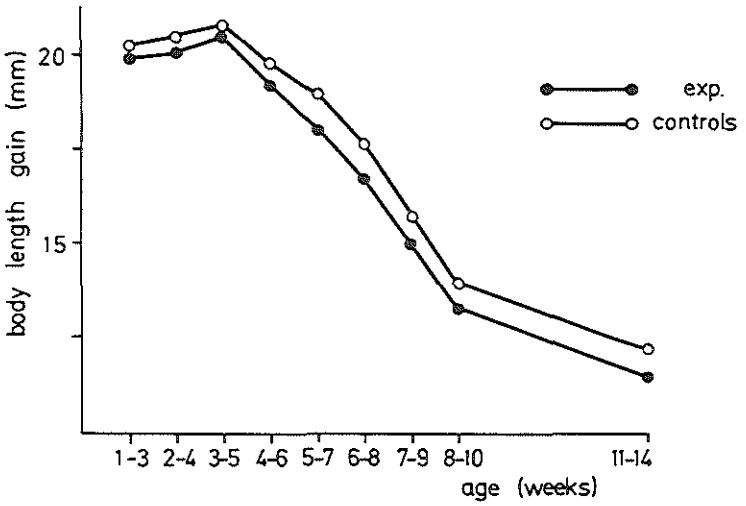
The initial growth retardation in our MTU-treated rats may be due to a decrease in pituitary STH secretion. Since growth rates were restored to approximately normal levels a few weeks later, this effect on the hypophysis is probably of a transitory nature in temporary hypothyroidism, even when this condition is present in the first two weeks of life. In fact from the age of 5 to 8 weeks the experimental animals grew faster than controls. The difference may be attributable to differences in the rate of skeletal maturation. The MTU-treated rats showed a considerable retardation of skeletal ossification (table 5, fig.6). Since progression of this ossification will have a depressive effect on the growth potential, the retardation of this process will also delay the decline in growth potential and so allow the animals to sustain a growth rate higher than normal for their chronological age. This is illustrated in fig.7A, where the body lengths are plotted against skeletal age instead of chronological age. It is clear that the curves for experimental and control animals are almost identical.

Quite different effects on growth patterns were obtained by neonatal treatment with thyroid hormones. Both treatments of this kind, the 14-day treatment with small doses (group B) and the 3-day treatment with large doses (group C), gave essentially the same picture, as can be seen in tables 3 and 4 and figures 2 - 5. Both groups growed at a persistently lower rate than their controls, which resulted in gradually increasing differences in length and weight with these normal animals. These results clearly show that the reduction in body weight found earlier by Hoskins (1927), Eayrs & Holmes (1964) and Swanson & Van

Fig.2. Influence of the three treatments on weekly length increments, represented by the running average of age periods of three weeks.
A. Treatment with MTU on days 2-15.
B. Treatment with 0,5 μg T3 on days 2-15.
C. Treatment with 40-50 μg T3 on days 2-4.



A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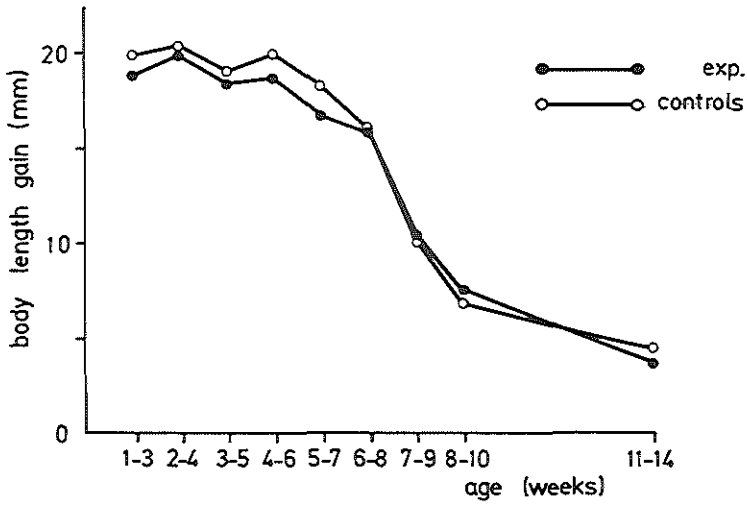
age (weeks)

B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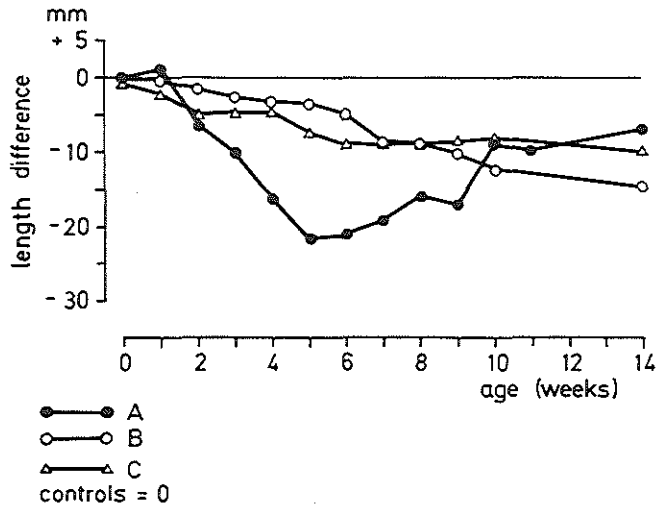


Fig.3. Mean differences in body length between the animals of all 3 treatment groups and their controls.
A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

der Werff ten Bosch (1955) after similar treatments, is of a persistent nature and related to a reduction in body length.

The results of the 3-day treatment (group C) demonstrate better than those of the B-group that there were two different effects to be distinguished. There was an immediately apparent effect during and shortly after treatment (table 4), followed by a beginning recuperation (week 3 and 4 for length, week 4 for weight); but from the fifth week onwards the animals were clearly smaller than normal again and growed persistently less even after. The immediate effect during treatment was greater in the C-group, receiving 130-150 μg in three days than in the B-group, receiving 7 μg in 14 days. The long-term effect, however, was markedly greater in the B-animals. This means that for the early effect dosage is more important, whereas for the long-term effect duration of treatment is more important than dosage. The first effect is probably mediated by a disturbance of metabolic processes, in the sense that amino acids are katabolized and therefore insufficiently available for protein synthesis. Therefore the immediate growth retardation may not be specific for hyperthyroidism, but may in fact be similar to the stunting of growth in neonatally malnourished rats. The nature of the effects of our treatments will be discussed in more detail in the general discussion.

TABLE 3. Mean length and weight gain ± SEM since day 2 in rats of treatment group B (T3 days 2-15, 8 experimental and 6 control animals).

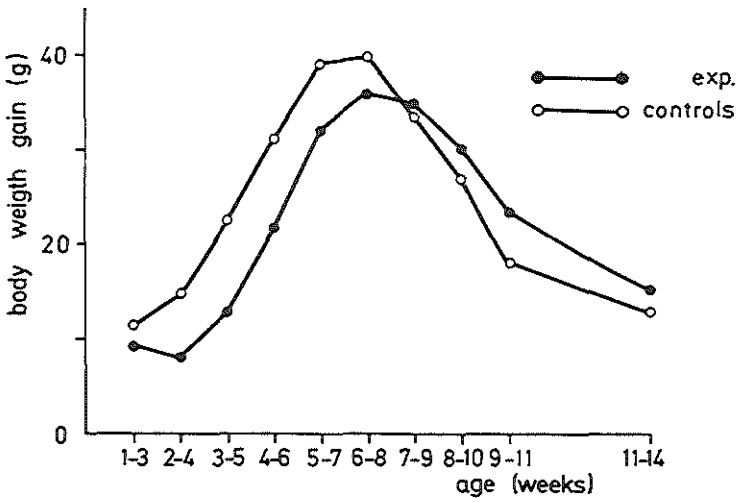
AGE WEEKS	LENGTH GAIN			WEIGHT GAIN		
	Exp.	Contr.	p	Exp.	Contr.	p
1	19,6 ± 1,3	19,5 ± 0,6	NS	8,3 ± 0,5	9,5 ± 0,3	0,047
2	36,6 ± 1,3	37,7 ± 1,2	NS	18,1 ± 0,8	21,3 ± 1,0	0,030
3	59,4 ± 1,4	61,3 ± 0,9	NS	34,4 ± 1,8	36,8 ± 1,9	NS
4	80,0 ± 1,4	82,5 ± 1,8	NS	63,6 ± 2,7	68,2 ± 3,5	NS
5	99,5 ± 1,5	102,3 ± 1,5	NS	97,0 ± 3,0	105,2 ± 4,6	NS
6	114,5 ± 1,6	120,2 ± 2,1	0,035	131,5 ± 3,3	142,8 ± 4,2	0,041
7	128,3 ± 0,9	136,2 ± 1,5	S	165,3 ± 4,4	183,5 ± 4,2	S
8	140,0 ± 1,7	148,0 ± 1,8	S	191,8 ± 5,3	221,3 ± 3,8	S
9	144,8 ± 1,6	154,5 ± 1,2	S	206,0 ± 4,8	246,8 ± 5,2	S
10	148,0 ± 1,4	160,0 ± 2,5	S	222,6 ± 5,6	267,5 ± 4,9	S
14	159,8 ± 1,5	173,7 ± 2,6	S	265,3 ± 7,5	325,7 ± 6,2	S

TABLE 4. Mean length and weight gain ± SEM since day 2 in rats of treatment group C (T3 days 2-4; 6 experimental and 5 control animals).

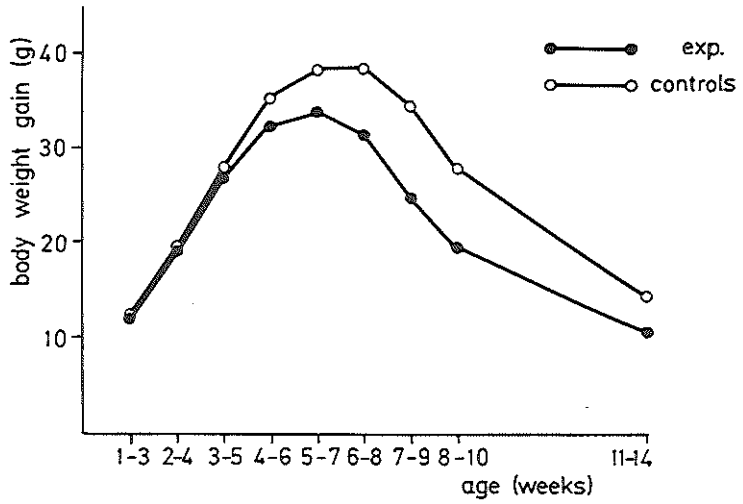
AGE WEEKS	LENGTH GAIN			WEIGHT GAIN		
	Exp.	Contr.	p	Exp.	Contr.	p
1	16,8 ± 1,4	18,0 ± 1,6	NS	5,8 ± 0,3	8,8 ± 0,4	S
2	36,8 ± 1,0	41,0 ± 0,7	S	19,3 ± 0,9	23,0 ± 0,7	S
3	56,2 ± 0,7	59,6 ± 1,8	0,076	33,3 ± 1,0	38,2 ± 0,8	S
4	76,7 ± 1,6	79,4 ± 2,1	NS	50,0 ± 2,0	56,2 ± 2,4	0,052
5	92,0 ± 1,5	98,4 ± 1,2	S	73,2 ± 4,5	93,8 ± 1,4	S
6	112,2 ± 1,5	120,0 ± 1,5	S	114,0 ± 3,8	131,6 ± 1,6	S
7	126,8 ± 1,4	134,2 ± 1,0	S	150,8 ± 4,1	172,2 ± 2,4	S
8	139,5 ± 1,9	146,6 ± 1,0	0,015	184,0 ± 4,6	211,6 ± 3,6	S
9	143,8 ± 1,3	150,2 ± 1,4	S	206,2 ± 4,3	230,0 ± 3,4	S
10	149,7 ± 1,0	155,0 ± 1,7	0,020	219,7 ± 4,5	245,8 ± 4,9	S
14	163,8 ± 1,3	173,2 ± 2,3	0,020	273,8 ± 6,7	319,6 ± 7,6	S

The long-term effect can be understood when again the data on skeletal maturation are taken into account (table 5, fig.6). Comparison of these data with those on growth reveals that while the B-animals showed a greater advancement in skeletal maturity than the C-group, their growth was also more retarded. The long-term effects on growth, that is the permanent reduction in length and weight (see also the autopsy data in table 7), are probably the result of premature epiphysial ossification which causes the animals to lose their growth potential at a faster rate than normal. In both groups the animals are very small not only for their chronological age, but even more so for their skeletal age (fig.7).

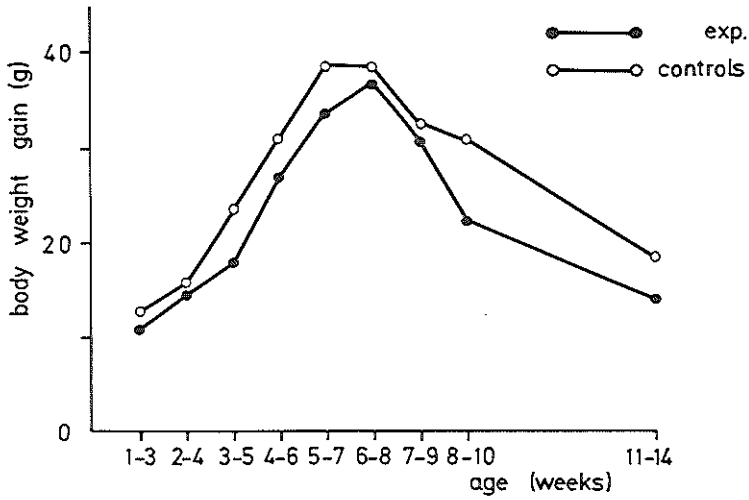
Fig.4. Influence of the three treatments on weekly weight increments, represented by the running average of age periods of three weeks.
A. Treatment with MTU on days 2-15.
B. Treatment with 0,5 μg T3 on days 2-15.
C. Treatment with 40-50 μg T3 on days 2-4.



A


body weight gain (g)
40
30
20
10
exp.
controls
1-3 2-4 3-5 4-6 5-7 6-8 7-9 8-10 11-14
age (weeks)

B


body weight gain (g)
40
20
0
exp.
controls
1-3 2-4 3-5 4-6 5-7 6-8 7-9 8-10 11-14
age (weeks)

C

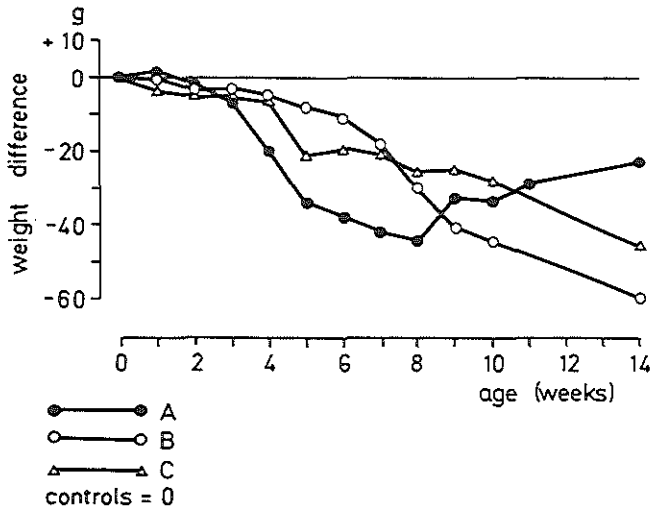


Fig.5. Mean differences in body weight between the animals of all 3 treatment groups and their controls.
A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

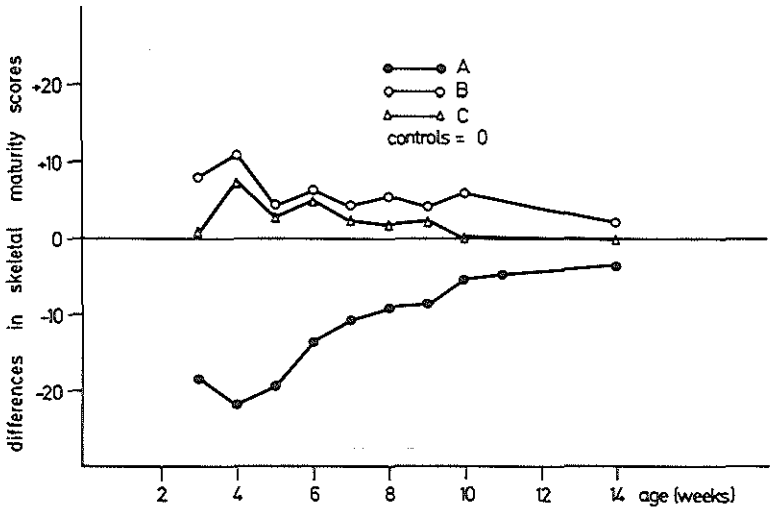
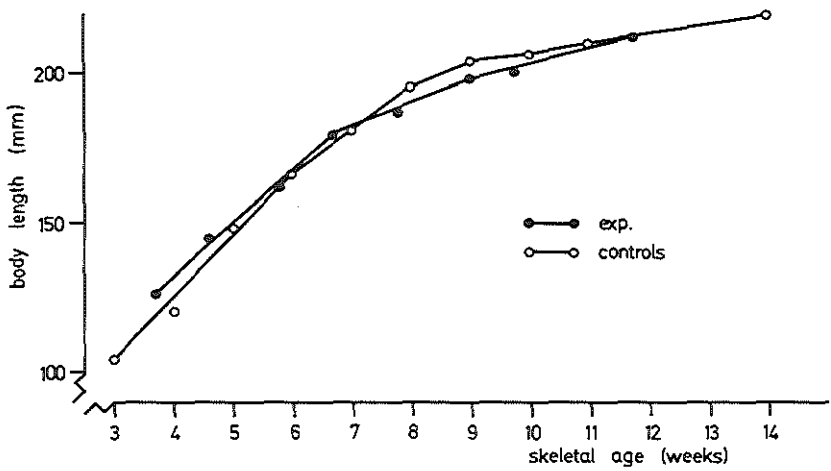


Fig.6. Mean differences in skeletal maturity scores between the animals of all 3 treatment groups and their controls.
A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

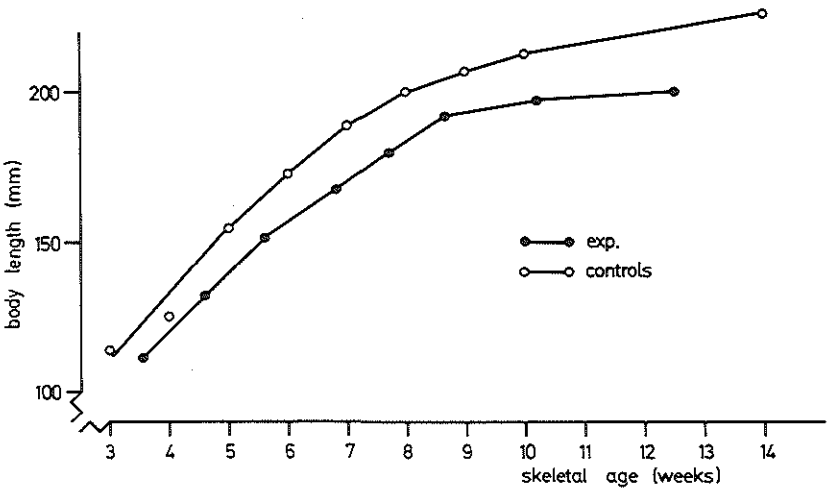
TABLE 5. Mean skeletal maturity scores ± SEM for all three treatment groups: A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

AGE WEEKS	TREATMENT A			TREATMENT B			TREATMENT C		
	Exp.	Contr.	p	Exp.	Contr.	p	Exp.	Contr.	p
3	67,2 ± 1,8	85,6 ± 1,3	S	102,8 ± 0,9	94,7 ± 0,3	S	100,5 ± 1,6	100,0 ± 0,3	NS
4	84,7 ± 2,2	106,6 ± 1,7	S	119,1 ± 0,4	108,0 ± 1,0	S	118,2 ± 1,9	110,6 ± 0,7	S
5	99,7 ± 2,1	119,2 ± 1,5	S	129,9 ± 0,5	126,1 ± 0,4	S	128,2 ± 0,7	125,4 ± 0,5	S
6	115,2 ± 2,7	128,9 ± 1,9	S	138,4 ± 0,4	132,4 ± 0,5	S	141,7 ± 0,9	136,0 ± 0,5	S
7	126,3 ± 2,0	137,0 ± 1,6	S	144,8 ± 0,6	140,9 ± 0,7	S	147,0 ± 0,5	144,3 ± 1,9	0,083
8	134,5 ± 1,7	143,7 ± 1,2	S	151,8 ± 0,4	146,3 ± 0,5	S	152,8 ± 0,2	151,0 ± 0,3	S
9	142,3 ± 1,1	151,1 ± 1,1	S	158,3 ± 0,6	154,1 ± 0,9	S	158,7 ± 0,5	156,4 ± 0,7	S
10	151,8 ± 1,8	157,4 ± 0,5	S	163,3 ± 0,3	157,3 ± 0,7	S	161,0 ± 0,4	160,8 ± 0,2	NS
11	156,2 ± 1,3	161,0 ± 0,7	S						
14	162,8 ± 0,1	166,7 ± 0,6	S	167,5 ± 0,3	165,7 ± 0,5	S	166,2 ± 0,3	166,2 ± 0,9	NS

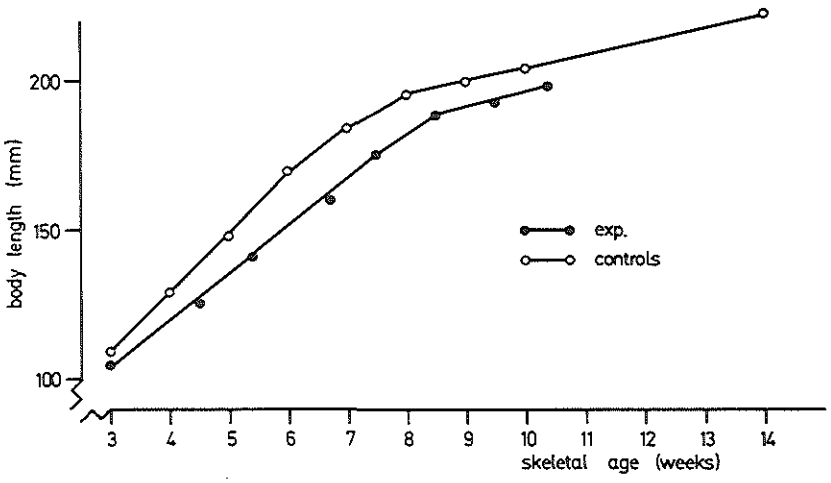
Fig.7. Influence of the three treatments on the relation of body length to skeletal age.
A. Treatment with MTU on days 2-15.
B. Treatment with 0,5 μg T3 on days 2-15.
C. Treatment with 40-50 μg T3 on days 2-4.



A


200
150
100
body length (mm)
exp.
controls
3
4
5
6
7
8
9
10
11
12
13
14
skeletal age (weeks)

B


200
150
100
body length (mm)
exp.
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3
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6
7
8
9
10
11
12
13
14
skeletal age (weeks)

C

Thyroid

It seems useful to consider the significance of the measures for thyroid activity used in this study before discussing the results that were obtained.

Thyroid weight. The activity of the thyroid gland is enhanced by TSH (thyroid stimulating hormone) from the hypophysis. When the thyroid is not able to produce sufficient amounts of thyroid hormones, TSH secretion will be increased. As TSH stimulates not only the synthesis and secretion of hormone by the thyroid, but also the growth of the gland, it will be enlarged in such cases. The best known cause of such "goitre" is dietary iodine deficiency such as in endemic cretinism. Another natural cause is a thyroidal enzyme deficiency. Experimentally such thyroid enlargement can be produced by administration of one of the several known antithyroid agents, drugs that block the synthesis of thyroid hormones. In contrast to this the thyroid will be very small and atrophic after prolonged administration of thyroid hormones, as such treatment suppresses the secretion of TSH by the pituitary. Apart from these extremes, variability in thyroid weight can be explained by differences in "hormonostat setting"; this means that the level of thyroid hormones in the blood that is sufficient to keep the pituitary from increasing its TSH secretion is variable.

Serum thyroxine level. The serum thyroxine level determination method used in this study was compared with the more commonly used serum PBI (protein bound iodine) determination by Kennedy & Abelson (1967). They obtained a very good correlation between the two in most circumstances. When, however, other iodinated compounds than thyroid hormones were added to the serum, PBI determination was affected seriously, whereas thyroxine determination was not. In this respect the latter method is certainly better, but it measures only thyroxine, free and protein-bound, not triiodothyronine. Altogether the thyroxine determination in serum may be regarded as a good measure for the availability of thyroxine to the tissues.

Effects of cold. Exposure to low environmental temperatures has been shown to increase the rate of both thyroid hormone secretion and peripheral loss of thyroid hormones and iodine. In rats exposed to cold, the thyroid activity as measured by the biological half-life of thyroidal radioiodine is increased (Van Beugen, 1960; Van Beugen & Van der Werff ten Bosch, 1961a,b). Stevens et al. (1955) found increased TSH levels in the blood of cold-exposed guinea-pigs, which indicates that the increased thyroid activity is due to increased TSH levels. In thyroxine-maintained thyroidectomized rats exposure to cold reduces PBI (Bondy & Hagewood, 1952; Kassenaar et al., 1956), which is an indication for increased peripheral disappearance of thyroxine. Kassenaar et al. (1959) indeed found an increased loss of injected ^{131}I labelled thyroxine in faeces and ^{131}I in urine after 24 hours of cold exposure in thyroidectomized rats; the loss in the urine consisted almost entirely of iodide, indicating an increased peripheral degradation rate of thyroxine. Galton & Nisula (1969) found

increased faecal loss of thyroxine in normal cold-adapted rats. As discussed by Van Beugen (1960), the increased TSH secretion in the cold causing increased activity of the thyroid could be explained in one of two ways: 1/ the increased peripheral loss of thyroxine in the cold causes a decrease in serum thyroxine levels, which by its negative feed-back action on the hypothalamo-pituitary system causes the pituitary secretion of TSH to be increased; 2/ the cold acts on the central nervous system which in turn causes the pituitary to increase its TSH output. Since effects of cold on the thyroid need about the same time to appear as effects of TSH, there remains no time for a hormonal feed-back effect. Hence the second hypothesis seems to be the right one (Van Beugen, 1960). Both thyroidal secretion and peripheral loss of thyroxine being increased by cold exposure and both kinds of effect being probably variable, it is not very surprising that studies on the effects of cold-exposure on blood PBI have yielded divergent results. Some authors reported unchanged blood PBI after exposure to cold during periods from a few days to several months, in different species (Ingbar et al., 1954; Stevens et al., 1955; Freinkel & Lewis, 1957; Galton & Nisula, 1969). Others found decreased PBI levels after different periods of exposure to cold (Ershoff & Golub, 1951; Bondy & Hagewood, 1952; Chevillard et al., 1967). Kassenaar et al. (1956) found elevated PBI levels in rats 24 hours after placement in the cold.

TABLE 6. Total serum thyroxine in μg/100 ml before and after one week at 5°C. Treatment groups: A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

TREATMENT		n	TOTAL SERUM THYROXINE μg/100 ml ± SEM	
			FIRST SAMPLE	AFTER ONE WEEK AT 5°C
A	exp.	5	4,70 ± 0,26	2,80 ± 0,17
	contr.	6	5,12 ± 0,03	3,15 ± 0,15
			NS	NS
B	exp.	6	4,77 ± 0,14	2,43 ± 0,13
	contr.	6	4,92 ± 0,30	2,85 ± 0,13
			NS	p = 0,013
C	exp.	5	4,92 ± 0,36	2,60 ± 0,15
	contr.	5	5,12 ± 0,10	2,66 ± 0,19
			NS	NS

The findings of the present study as given in table 6, show that a considerable decrease in serum thyroxine levels occurred in all animals after one week's exposure to 5°C. This could be due to an increased portion of triiodothyronine

in the thyroid hormone release of the thyroid gland, which very potent hormone is not measured by the method used in this study. The fact that in several earlier studies decreased PBI values were obtained makes it more likely that the increase in thyroid activity has been relatively low, and the peripheral loss of thyroxine relatively high.

Effects of neonatal hypothyroidism. Neither before nor after cold exposure were thyroxine levels of the MTU-treated rats different from those in control animals (table 6). The relative weight of the thyroid gland was greater in the experimental animals than in controls (table 8). The combination of increased thyroid weight and normal thyroxine levels suggests that the neonatal hypothyroidism changed the setting of the feedback mechanism in such a way that more TSH is necessary to induce the thyroid gland to maintain adequate levels of thyroid hormones in the blood.

In a similar experiment Bakke et al. (1970) treated rats with PTU (propylthiouracil) either prenatally or neonatally or both. Examining the animals at ages from 24 to 147 days they found a persistent enlargement of the thyroid gland (as in the present study), but also diminished serum thyroid hormone levels (which contrasts with the findings reported here). These authors also measured pituitary TSH content in three different groups of animals: 1/ without further treatment; 2/ after administration of 0,05% PTU in the drinking water during 10 days; 3/ after similar PTU administration accompanied by an injection of 20 μg T4/100 g body wt. on the last day, in order to measure the net TSH synthesis over the following 24 hours. They found for perinatally PTU-pretreated rats (versus controls) a TSH content in mU/gland of 326(116), 183(54) and 238(244). Their conclusion was that the synthesis of TSH was diminished in the perinatally PTU-treated rats and, consequently, that it was very unlikely that the enlargement of the thyroid reflected an elevated serum TSH level. This hypothesis meets with two great difficulties. In the first place it does not offer an explanation for the heavier thyroid gland. In the second place it is very unlikely, that with a decreased TSH synthesis the TSH content would be so dramatically increased from 116 to 326 as it was, unless the release from the pituitary would have been decreased to an even greater extent, which is very unlikely in view of the decreased T4 levels in the blood and the considerable depletion found after the PTU-administration. Therefore, it seems more likely that the increased thyroid weight indeed reflected higher TSH levels, induced by a decreased sensitivity of the thyroid to TSH. The lower increase in TSH content after a blockade of TSH release by administration of T4 does not necessarily reflect a lower rate of TSH synthesis, but more likely a lower sensitivity of the hypothalamo-pituitary system to the feed-back action of thyroxine. PTU and other thiouracil derivates are known not only to block thyroid hormone synthesis, but also to counteract thyroxine at the target organ level, including the feed-back action on the hypothalamo-pituitary axis (Morreale de Escobar & Escobar del Rey, 1968).

Perinatal administration of these antithyroid agents could very well have caused a persistent decline in the sensitivity of the hypothalamo-pituitary system to thyroxine. Such a changed setting of the "thyreostat" at both levels could account for a certain variability in the net results as measured by determination of circulating thyroid hormone levels. Another possible explanation for the discrepancy between the thyroid hormone levels found by Bakke et al. (1970) and those reported here may be provided by the difference in antithyroid compounds used. PTU is 11 times more potent than MTU in the rat, as measured by a method based upon weight of the thyroid and its iodine concentration (Astwood et al., 1945), so that the effective dose given by Bakke and his associates may have been greater.

Effects of neonatal hyperthyroidism. In the groups treated neonatally with T3, no differences in relative thyroid weight were found (table 8). The serum thyroxine levels (table 6) were not different from control values before cold exposure, but after 7 days at 5°C the rats of experiment B (but not those of C) had a slightly but significantly lower serum thyroxine level than control animals.

The findings on thyroid weight are in conformity with those of Eayrs & Holmes (1964), who collected their results at day 100, after similar neonatal treatments with T3. Bakke & Lawrence (1966), however, obtained a decrease in relative thyroid weight on day 42, after neonatal treatment with T4. In comparing their own results with those of Eayrs & Holmes (1964), these authors suggest that their use of T4 instead of T3 was the cause of the difference between the results. This suggestion may be correct, but another possible explanation might be found in the difference in age when examined. Swanson & Van der Werff ten Bosch (1965) found no persistent effects of neonatal treatment with T3 and T4 on thyroid activity, as measured by determination of radioiodine uptake and half-life of thyroidal radioiodine. This observation supports the findings reported in the present study concerning the unchanged thyroxine levels in normal environmental temperatures.

The greater decrease in serum thyroxine levels after exposure to cold in the B-animals (absent in the C-group), probably means that the longer duration of the neonatal T3-treatment in the B-group, as compared with the greater dose given during only three days in the C-group, may have been decisive in affecting structures in the hypothalamo-pituitary system that are responsible for the increased pituitary TSH output in the cold. The finding of Bakke & Lawrence (1966), who reported a decreased ability of the hypothalamo-pituitary system to produce an increase in TSH secretion, after treatment with PTU in neonatally T4 pretreated rats, seems to support this view.

Conclusion. With regard to the question whether or not the neonatal treatments applied in this study did provoke persistent changes in thyroid function that may be held responsible for the effects on physical and behavioural

TABLE 7. Autopsy data. Brain wt. refers to total brain, seminal vesicles inclusive coagulation glands.
Treatment groups: A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

TREAT-MENT	n	AGE AT AUTOPSY days	BODY WT. g	BODY LT. mm	BRAIN WT. mg	THYROID WT. mg	WT. 2 ADRENALS mg	WT. 2 TESTES mg	WT. 2 SEM. VES. mg
A exp.	5	231,6 ± 1,0	407,4 ± 13,6	236,2 ± 1,8	1890,6 ± 48,0	56,0 ± 5,4	53,3 ± 1,8	3723,4 ± 89,7	506,4 ± 15,9
contr.	6	232,0 ± 0,9	432,0 ± 4,3	237,7 ± 1,2	2059,7 ± 46,2	46,3 ± 1,1	57,2 ± 1,7	3278,3 ± 50,3	480,7 ± 16,7
			NS	NS	p = 0,026	p = 0,075	NS	S	NS
B exp.	6	218,0 ± 12,5	329,0 ± 17,0	224,7 ± 2,6	1827,5 ± 38,8	40,8 ± 3,6	51,0 ± 1,2	2059,5 ± 77,7	436,3 ± 14,7
contr.	6	218,0 ± 12,5	416,8 ± 15,6	238,5 ± 2,1	2084,0 ± 23,5	48,0 ± 2,0	57,7 ± 1,1	2941,7 ± 66,0	467,3 ± 21,0
			S	S	S	p = 0,066	S	S	NS
C exp.	5	224,0 ± 2,5	360,8 ± 10,5	230,4 ± 2,4	1790,8 ± 32,4	53,0 ± 3,9	52,2 ± 1,9	2667,2 ± 97,9	587,8 ± 52,2
contr.	5	226,0 ± 2,5	416,4 ± 7,9	240,4 ± 0,5	1878,2 ± 61,0	60,6 ± 5,2	62,4 ± 2,6	3059,0 ± 55,4	573,6 ± 31,6
			S	S	NS	S	NS	S	NS

TABLE 8. Autopsy data. Organ weights relative to body weight.
Treatment groups: A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2.4

TREAT-MENT	BRAIN WT. mg/100 g BWT.	THYROID WT. mg/100 g BWT.	ADRENALS WT. mg/100 g BWT.	TESTES WT. mg/100 g BWT.
A exp.	463,6 ± 7,7	13,8 ±1,4	13,2 ±0,2	915,0 ±12,8
contr.	477,3 ± 13,0	10,8 ±0,3	13,2 ±0,4	759,5 ±15,0
	p = 0,063	p = 0,041	NS	S
B exp.	560,0 ± 18,2	12,4 ±0,7	15,7 ±0,6	631,7 ±13,4
contr.	503,0 ± 17,4	11,5 ±0,5	13,9 ±0,4	708,0 ±15,6
	p = 0,032	NS	p = 0,04	S
C exp.	497,8 ± 7,8	14,8 ±1,5	14,5 ±0,5	739,4 ±10,2
contr.	451,2 ± 15,4	14,5 ±1,0	15,0 ±0,4	735,4 ±13,6
	p = 0,028	NS	NS	NS

development seen in the adult rats, the conclusion seems justified that this is not the case, on the following grounds:
1/ neither of the treatments caused a change in adult serum thyroxine levels at normal environmental temperatures;
2/ the differences in thyroxine levels after cold exposure in the B-animals were not very great and moreover not very relevant in comparison with the levels before cold exposure, because in the studies on physical and behavioural development the measurements were taken from animals that had been kept at room temperature all the time.

Adrenals

The only significant effect of the treatments on adrenal weight at autopsy was found in the B-animals (tables 7 and 8). The adrenals were significantly smaller in the experimental animals, but slightly heavier when calculated per 100 g body weight. Apparently the adrenals were affected to a lesser extent than the whole body. The same tendency can be observed in the other treatment groups, although no significant differences were obtained. These findings do not provide any indication of persistent general pituitary damage.

Testes and Seminal vesicles

Effects of neonatal hypothyroidism. The MTU-treated rats had absolute and relative testis weights that were considerably higher than those of control animals at autopsy (tables 7 and 8). The fact that no significant differences were found in the weights of the seminal vesicles (table 7) makes it very unlikely that the differences in testis weight were accompanied by significant changes in androgen secretion. A complementary experiment was done in

order to establish whether or not such adult differences in testis weight are already present before puberty. Two litters of 10 rats were treated with MTU as in the main study. The animals of these two litters were killed at day 16 and 42 respectively. Body weights and testis weights are given in table 9, together with the results of the adult animals from table 7 and 8. Evidently the larger testes in the experimental rats do not develop before puberty.

Although an overwhelming amount of evidence has been collected that thyroidectomy prevents normal development and function of the testis in many species of birds and mammals, including the rat (Gomes, 1970), reports on the consequences of temporary neonatal hypothyroidism for testicular development and function are not available. Bakke et al. (1970), in their study on the effects of perinatal treatment with PTU, found that in female rats the occurrence of vaginal opening and first vaginal oestrus were significantly delayed, while the cycle length was significantly prolonged. Meites & Chandrashaker (1949) treated rats with MTU from day 21-30 after birth till day 40, and found then an enhanced response of the testes to PMS (pregnant mare serum): weight of the

TABLE 9. Body weights, testes weights and relative testes weights at different ages in neonatally MTU-treated rats.

AGE	GROUP (n)	BODY WT. g	WEIGHT 2 TESTES mg	WEIGHT 2 TESTES mg/100 g
16 d	Exp. (5)	25,8 ± 1,0	78,6 ± 6,9	302,6 ± 18,4
	Contr. (5)	32,0 ± 1,3	116,0 ± 11,9	358,4 ± 20,5
		p = 0,028	p = 0,048	p = 0,048
6 w	Exp. (5)	121,2 ± 4,6	1258,8 ± 59,3	1033,4 ± 17,0
	Contr. (5)	156,2 ± 3,0	1587,0 ± 36,7	1016,0 ± 11,2
		S	S	NS
33 w	Exp. (5)	407,4 ± 13,6	3723,4 ± 89,7	915,0 ± 12,8
	Contr. (6)	432,0 ± 4,3	3278,3 ± 50,3	759,5 ± 15,0
		NS	S	S

testes and seminal vesicles increased more than in controls. Hara (1963) thyroidectomized rats on day 21, and found subnormal testis weights at day 100. In his study the histological examination of the seminiferous tubules revealed that germ cells remained in the spermatocytic stage. The fact that these effects could be prevented by simultaneous T3 medication led this athor to conclude that thyroid hormones are necessary for spermatogenesis.

Effects of neonatal hyperthyroidism. The T3-treated rats of the B-group showed testis weights that were absolutely and relatively lower than in control animals (tables 7 and 8). The effect on relative testis

weights was completely absent in the animals of the C-group. Neither of the two methods of T3 treatment had an effect on the weight of the seminal vesicles (table 7). With regard to the effects of experimental hyperthyroidism on testicular development and function, earlier findings are contradictory (Gomes, 1970), and almost all of them concerning treatment after the neonatal period. Bakke et al. (1969) observed a similar delay of first oestrous day and lengthening of oestrous cycles in neonatally T4-treated rats as they did after neonatal treatment with PTU (Bakke et al., 1970). DaCosta & Carlson (1933) reported delayed secual maturation and lower adult testis weights after thyroid feeding from ages between 18 and 21 days. Meites & Chandrashaker (1949) fed rats with desiccated thyroid from ages varying from 21 to 30 days; they found a slightly diminished response to PMS as measured by the weights of testes and androgen target organs.

Discussion. With the evidence from earlier reports available at this moment it is very difficult to identify the mechanism by which the effects on testis weight obtained in the present study were brought about. There are three main possibilities to be discussed: 1/ the treatments caused a change in pituitary function of either a persistent or a transitory nature; 2/ the treatments have exerted a direct influence on the testes, changing their sensitivity to gonadotropins; 3/ the effects on testis weights are secondary to the effects on general body growth.

The hypothesis that the enlargement of the testis of the MTU-treated rats was caused by increased pituitary gonadotropin secretion seems to be inconsistent with earlier reports that neonatal treatment with PTU causes delayed first oestrous day and lengthening of oestrous cycles in female rats (Bakke et al., 1970), and that thyroidectomy produces no increase in the gonadotropic activity of plasma in rats (Contopoulos et al., 1958). The findings of the present study on other endocrine effects of early MTU treatment do not provide any evidence for persistent changes in pituitary function. The fact that no difference in the weights of the seminal vesicles was found in any of the treatment groups makes it doubtful that the secretion of gonadotropins, at least of ICSH (interstitial cell stimulating hormone) would have been persistently affected. Even a temporary increase in gonadotropic activity of the hypophysis before puberty is very unlikely in view of the data on testicular weight at the ages of 16 days and 6 weeks as obtained in the complementary study (table 9).

The view that the reduction of testis weight in the T3-treated rats of the B-group could be caused by persistent pituitary changes meets with a complete lack of evidence for such persistent pituitary damage in the results on the weights of other endocrine organs and seminal vesicles in both B- and C-group.

Support for the second hypothesis, that a damage in testicular sensitivity to gonadotropins was underlying the differences in testis weights, is provided by the findings of Meites & Chandrashaker (1949) mentioned above. The failure of

the rats in this study to show differences in the weights of the seminal vesicles would indicate that this effect was of a persistent nature only in the sensitivity to FSH and that a changed sensitivity to ICSH was either temporary or not present at all.

The absence of differences in testis weights in the C-group would mean that in spite of the large dose given to these rats, the hyperthyroid state has been of too short duration and was ended before an effect upon the testes could be established. The results of Meites & Chandrashaker (1949) show that treatment as late as during the fourth and fifth week after birth is successful, whereas there is no evidence available that treatment during only the first few days after birth does the same. Hence it seems possible that the period during which hypo- and hyperthyroidism exert such influence on the sensitivity of the testes to gonadotropins does not commence before the second week after birth.

The third hypothesis, that the effects on testis weights were secondary to the effects on body growth, finds support in the results of this study itself as well as in the results of the complementary study mentioned above. The latter show clearly that MTU-treated rats do not yet have larger testes than controls at day 42. This could mean that the larger mature testes in the experimental animals were brought about by the slower decline of their general body growth rate as compared with control data from about 6-7 weeks onwards (fig.4A). The pubertal growth spurt of the testis would then coincide with a more rapid general growth rate than in controls, which might have added to the growth of the testes. In the same way the low testis weights of the B-animals could be explained by their persistently lower growth rate (fig.4B). True enough this difference in growth rate seems to be smaller in the C-animals (fig.4C), but the complete absence of differences in relative testis weights in this group cannot be accounted for along these lines. Evidence against this hypothesis is also provided by the finding of Widdowson & McCance (1960) (who compared growth patterns of body and organs in rats suckled in small and big litters) that testis weights were proportional to body weights in fast growing and slow growing rats. The reasonable assumption that puberty was delayed in the MTU-treated rats (Bakke et al., 1970), is not in favour of this third hypothesis, because in that case the growth rates of experimental and control animals could very well have been similar during puberty. The assumption that delayed puberty might have given the testes of the experimental animals a higher initial weight at the onset of puberty could possibly account for the large testes of the MTU-treated rats, but certainly not for the small testes of the T3 treated rats of the B-group, because, according to earlier reports (DaCosta & Carlson, 1933; Bakke et al., 1969, 1970, puberty in these animals has been delayed rather than advanced.

The available evidence seems to be in favour of the view that neonatal deviations from the euthyroid state affect the sensitivity of the testes to FSH, hypothyroidism making it increase and hyperthyroidism making it decrease.

More evidence will be required before reliable conclusions can be drawn.

Brain

Brain weights were reduced in all three treatment groups. In consequence of T3 administration this reduction in weight was much less in the brain than in the whole body (tables 7 and 8). These findings accord with earlier reports. Neonatal hypothyroidism brought about by neonatal thyroidectomy is followed by a reduction in brain weight at later ages due to impairment of cell growth: protein synthesis as measured by determination of protein/DNA ratio or amino acid incorporation is diminished (Geel & Timiras, 1967, 1970, 1971; Geel et al., 1967; Pasquini et al., 1967; Balász et al., 1971). Mitotic activity is not impaired, since the cellular density, as measured by determination of DNA per unit of wet tissue weight, is above normal (Geel & Timiras, 1967; Pasquini et al., 1967; Balász et al., 1971). Both decreased protein/DNA ratio and increased cellular density were already present on day 5 in the experiments of Pasquini and her coworkers (1967). Neonatal excess of thyroid hormones reduces brain weight by reducing the number of normal-sized cells (Balász et al., 1971). It is probable that the thyroid hormone excess forces cells into premature differentiation, thus causing them to lose their mitotic activity too rapidly. Evidence for this view has accrued from in vitro studies by Hamburgh (1966, 1968).

BEHAVIOURAL DEVELOPMENT

Material and methods

Nervous system maturation and automatic behaviour

All animals used in the study of physical growth were checked daily for the presence of open eyes, placing reaction and righting reflex from day 8 until all three criteria were present in all animals.

The eyes were considered to be open on the first day both eyeballs were visible.

The placing reaction was taken for present when the animal would put both forepaws on a metal rod placed horizontally against its nose within two seconds, if being lifted in the air between two fingers in its flanks, legs down.

The criterion for the presence of the righting reflex was the ability to land on four feet when dropped back down from a standard height of approximately 30 cm above a layer of cotton wool.

Locomotor activity

From each of six litters from each treatment group one experimental and one control animal were randomly chosen on the day of weaning for activity measurements.

The apparatus chosen for the measurement of locomotor activity was a "residential plus-maze" as first designed and described by Barnett and his associates in Glasgow (Barnett & Smart, 1965; Barnett et al., 1966, 1971). In this study a slightly modified type was used which is illustrated in fig.8.

On the day of weaning the littermate pair of rats was placed each in a plus-maze for an adaptation period of three days and a subsequent registration period of seven days. In order to avoid any bias by disturbances during the day, the average number of visits to the arms during the 10-hour periods of darkness between 21.00 h and 07.00 h was taken as measure for activity. After this first registration at about one month of age the animals were placed in a normal laboratory cage again for the next two months. At the age of three months the

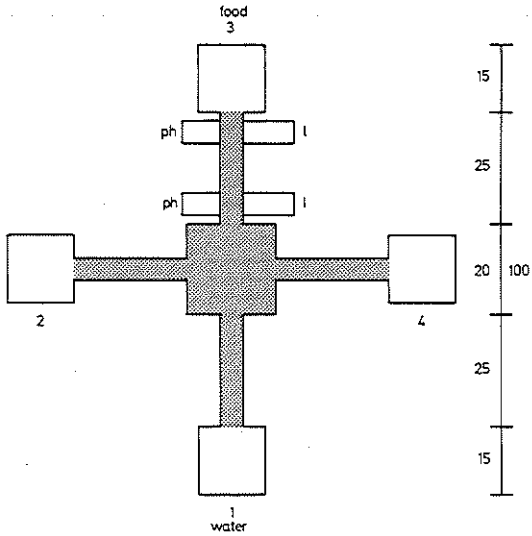

Fig.8. Schematic ground plan of plus-maze as used in these experiments. The hatched areas, including the central nestbox and the narrow part of the four arms, have a metal top, the four goalboxes have transparent tops. The arms have grid bottoms and are 10 cm high, the nestbox has a removable metal bottom and is 20 cm high, it has sawdust on the bottom. All four arms have two lamps (l) and two phototransistors (ph) as indicated for arm 3. The lamps have infrared filters. When the animal enters an arm and interrupts the first infrared beam, a circuit is primed. Interruption of the second beam will then induce a printer to print out the number of the arm and the time in hours, minutes and seconds. When the rat goes back a print will be made in the same way on interruption of the beam nearest to the nestbox. Each maze is provided with its own source of illumination, a 20 W. fluorescent tube placed diagonally above the maze, thus lighting the four goalboxes in the same way. Horizontal dimensions are given in the figure in cm.

same procedure was repeated. All data of nights during which bias by failure of an apparatus was found or suspected, were discarded in calculating averages for each animal.

Learning ability

A number of animals not used for activity measurements, but from the same litters, were subjected to a test for learning ability. The test chosen was the "closed field test" first described by Hebb & Williams (1946). The maze used in this test is generally known as the "Hebb-Williams maze". The apparatus as well as the procedure used in these studies were similar to those described by

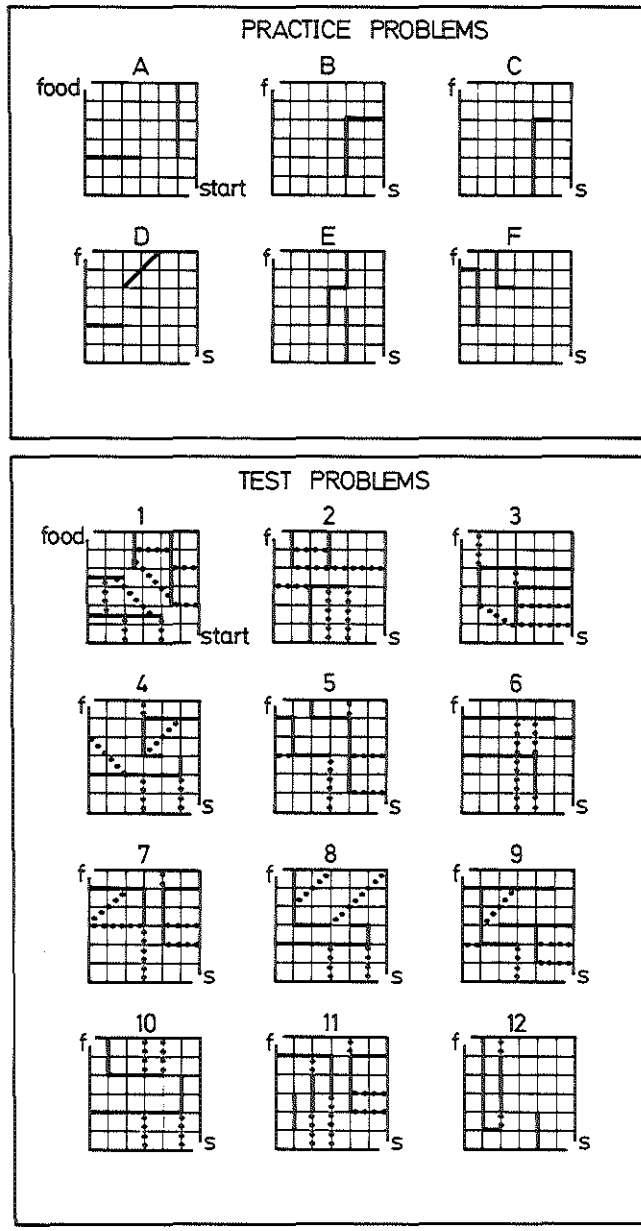

Fig.9. The problems of the closed-field test. For explanation see text. (after Rabinovitch & Rosvold, 1951).

Rabinovitch & Rosvold (1951), and by Barnett (1963).

The maze is essentially a wooden box of 75 x 75 cm, 10 cm high. The walls are painted black, the floor is painted white with 36 squares of 12,5 x 12,5 cm outlined in black. The maze has a grid top and two boxes, which can be attached to two opposite corners of the maze. These boxes are provided with a tip-up transparent lid and a trap-door giving access to the maze and are used as start- and goalbox respectively. With the maze are a number of black wooden barriers 10 cm high and 12,5, 25, 37,5, 50 and 67,5 cm long. By placing some of these barriers in different arrangements on the black lines in the maze, a variety of maze-problems can be created. The problems as described by Rabinovitch & Rosvold are illustrated in fig.9.

The animals were subjected to a food deprivation schedule during the entire procedure described below, getting food during only one period per day in the goalbox of the maze between runs and in special cages immediately after completing their runs for the day.

The procedure itself was divided in three stages:

1. An "adaptation" period of three days, during which the animals were left in the maze in small groups during an hour with food in the goalbox in order to get accustomed to the maze and the location of the food.
2. A "practicing" period, during which the rats had to run the practice problems A-F. This was done individually, one problem per day, until all rats of the group had met the criterion for beginning the real test. This criterion meant completing 9 runs on the same problem within a total time of 60 seconds. This took about a week on the average.
3. The testing period of 12 days. Each day the rats had to run one test problem 8 times in succession. Now time was not important any more for the score on the test; only the number of errors was counted. Each time a rat entered an error zone (in fig.9 demarcated with dotted lines) with both forepaws was counted as an error. The total number of errors made on 12 x 8 runs was used as measure in this study.

Results and discussion

Nervous system maturation and automatic behaviour

The data listed in table 10 on the emergence of placing reaction and righting reflex and on the opening of the eyes are in accordance with earlier reports that such and other aspects of maturation are delayed by neonatal hypothyroidism and advanced by neonatal hyperthyroidism (table 1). Since several authors obtained such results not only by means of treatment with MTU or T3, but also by thyroidectomy or treatment with T4, such changed timing of maturation can be considered as a reliable indication of abnormal thyroid state. Consequently the day of eye opening was used in this study as a check on the presence of hypo- and hyperthyroidism in the litters used for the behavioural studies discussed below.

TABLE 10. Mean age in days ± SEM at first appearance of open eyes, righting reflex and placing reaction.
Treatment groups: A. MTU 2-15; B. T3 days 2-15; C. T3 days 2-4.

TREAT-MENT	n	EYES OPEN DAY	RIGHTING REFLEX DAY	PLACING REACTION DAY
A exp.	10	16,9 ± 0,4	20,8 ± 0,4	22,3 ± 0,3
contr.	10	14,9 ± 0,4	15,8 ± 0,4	19,1 ± 0,6
		S	S	S
B exp.	9	10,8 ± 0,3	11,8 ± 0,5	16,8 ± 0,5
contr.	11	13,9 ± 0,3	14,4 ± 0,4	17,6 ± 0,4
		S	S	NS
C exp.	8	12,8 ± 0,3	14,5 ± 0,6	17,6 ± 0,5
contr.	12	14,6 ± 0,2	15,8 ± 0,3	19,6 ± 0,6
		S	p = 0,019	S

Locomotor activity and learning ability

The data on locomotor activity (tables 11 and 12, fig.10) show that only the MTU-treated rats had abnormal activity levels at one month of age. Probably this low activity has to be attributed to the delayed maturation of their nervous system. The fact that the existence of hypothyroidism has been reported to lower activity levels in the rat (Hall & Lindsay, 1938; Mann, 1945; Richter, 1933) suggests the possibility that the animals may have been not yet quite recovered from the treatment. The short duration of the effectiveness of thiouracil derivatives in clinical use (Astwood, 1965), however, makes this explanation unacceptable.

The most striking effects of the treatments on activity were seen two

TABLE 11. Activity between days 25 and 32 after birth.
Treatment groups: A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

TREAT-MENT	n	MEAN NR. OF EXCURSIONS PER NIGHT (21.00–07.00 h) AGE 1 MONTH				
		arm 1 water	arm 3 food	arm 2	arm 4	TOTAL
A exp.	5	14,4	12,8	10,0	11,1	48,3
contr.	6	22,7	26,8	20,6	21,3	91,3
		S	S	S	p = 0,015	S
B exp.	5	27,4	33,5	25,2	28,7	114,5
contr.	6	26,2	30,5	24,4	21,8	102,9
		NS	NS	NS	p = 0,015	p = 0,074
C exp.	6	27,6	31,3	30,2	26,6	115,6
contr.	6	28,0	35,1	23,2	23,3	108,2
		NS	NS	p = 0,066	NS	NS

TABLE 12. Activity three months after birth.
Treatment groups: A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

TREAT-MENT	n	MEAN NR. OF EXCURSIONS PER NIGHT (21.00-07.00 h) AGE 3 MONTHS				
		arm 1 water	arm 3 food	arm 2	arm 4	TOTAL
A exp.	5	22,8	16,4	17,3	20,6	77,1
contr.	5	13,6	11,1	12,9	12,0	48,4
		p = 0,048	NS	p = 0,075	p = 0,016	p = 0,048
B exp.	6	21,6	20,2	19,2	20,7	81,8
contr.	6	11,9	9,4	10,2	11,4	42,8
		S	S	S	S	S
C exp.	6	24,5	22,5	26,4	25,7	99,0
contr.	5	16,2	9,0	15,1	14,3	54,6
		p = 0,015	S	S	S	S

months later. All normal control rats showed a decrease in activity to about 50% or less of the levels measured one month after birth. A similar decline with age in the activity of rats was reported earlier by Jones et al. (1953). Although they recorded the activity in a "running wheel" and studied no rats younger than 51

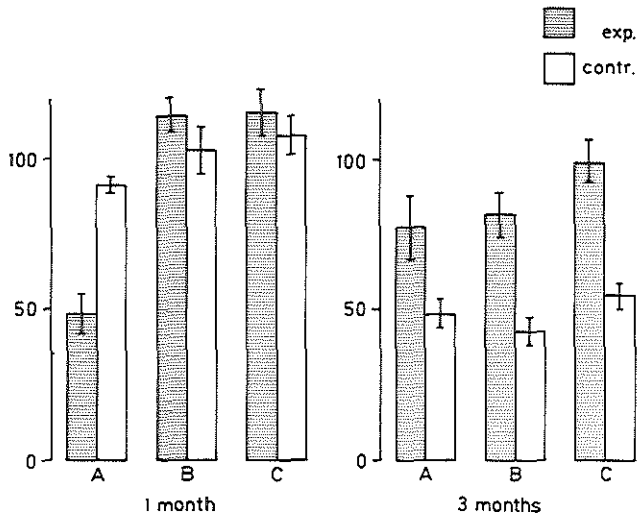

Fig.10. Influence of treatments on bodily activity at 1 and 3 months after birth. Vertical bars indicate SEM.
A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

days, their findings seem to point in the same direction as the results of the present study. This decline was not present in the animals of any of the experimental groups, which all displayed activity at levels far above normal. The only earlier observation on such hyperactivity after neonatal hyperthyroidism was reported by Schapiro (1968), who used an entirely different and not well comparable method. He observed elevated ambulation scores in an "open field test" in 5-6 weeks old rats treated neonatally with T4. This could mean, if anything, for our plus maze recordings that the hyperactivity, not yet present at 4 weeks after birth, develops soon afterwards. Reports on the effect of early hypothyroidism on later activity are not available, except for one remark of Scow & Simpson (1945), that neonatally thyroidectomized rats were "more active than expected". It is not clear whether their hypothyroid rats were actually more or less active than normal controls.

The finding that neonatal treatments of opposite nature cause the same abnormality of adult locomotor activity is the more interesting, because the data on learning ability give a different picture (table 13, fig.11). Neonatal treatment with thiouracil caused the animals to make more errors in the Hebb-Williams maze, whereas the T3-treatments had no effect. Eayrs (1961, 1964), however, obtained increased error scores after both kinds of neonatal treatment with the same method as used in the present study. The origin of this discrepancy is difficult to evaluate. The only obvious differences were in the strains of rats used

TABLE 13. Mean number of errors ± SEM made in the closed-field test.
Treatment groups: A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

TREAT-MENT	n	MEAN AGE WHEN TESTED DAYS	MEAN NR. OF ERRORS HEBB-WILLIAMS MAZE
A exp.	5	132 ± 3,4	211,6 ± 17,4
contr.	6	133 ± 3,1	150,0 ± 13,3
			p = 0,015
B exp.	6	158,5 ± 12,7	154,3 ± 12,0
contr.	6	158,5 ± 12,7	146,8 ± 8,2
			NS
C exp.	5	163,0 ± 3,0	173,6 ± 17,8
contr.	5	165,0 ± 3,0	163,2 ± 13,6
			NS

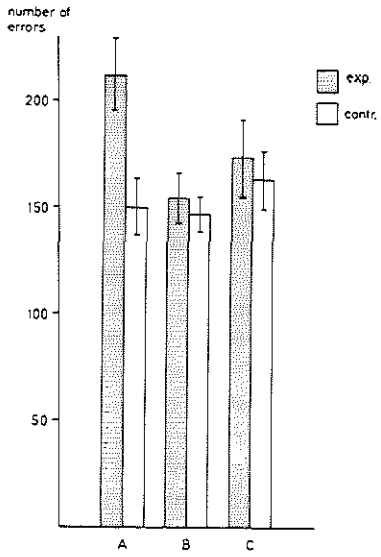

Fig.11. Influence of treatments on error scores in the closed-field test. Vertical bars indicate SEM.
A. MTU days 2-15; B. T3 days 2-15; C. T3 days 2-4.

and the ages at which the animals were tested. Eayrs tested them about day 100, whereas in this study testing of the T3-treated rats began approximately two months later. It might be interesting to test such rats at different ages between one and seven or eight months in order to investigate the possibility that the

impairment of learning ability found by Eayrs after treatment with thyroid hormones, is not completely irreversible but gradually declines with age.

In discussing the mechanisms by which the behavioural abnormalities could have been brought about, several possibilities will have to be considered. Especially with regard to the hyperactivity some persistent change in the endocrine state might have been the underlying cause, as several hormones are known to affect locomotor activity, particularly the thyroid hormones and gonadal steroids. Apart from the fact that no evidence for major changes in circulating thyroid hormone levels was found, the hypothesis that the presence of such changes could cause hyperactivity is hardly tenable in view of reports which established that gross deviations from the euthyroid state in whatever direction will depress locomotor activity in adult rats and never make it increase, whereas minor deviations have no noticeable effect (Mann, 1941, 1945; Richter, 1933). As mentioned in the previous chapter, testis weight was considerably affected in the treatment groups A and B, but not in group C. This alone makes it very unlikely that the hyperactivity in all treatment groups would have been caused by these changes. Moreover the normal weights of the seminal vesicles give no support at all for the supposition that the secretory activity of the testes was abnormal. The data on adrenal weights do not give any indication of the kind either.

It seems more likely that the underlying mechanism(s) can be found in persistent changes of brain function. As can be seen in table 1, many structural and chemical abnormalities of the brain can be brought about by manipulating thyroid hormone levels in early infancy. The brain goes through a period of very rapid development until the third week after birth, which makes it very vulnerable. This does not necessarily mean that such gross structural changes had occurred and, if they had, that they were actually responsible for the abnormal behaviour of our animals. In fact malnutrition from the third or fourth week onwards followed by a period of rehabilitation, caused a similarly marked hyperactivity in adulthood in the experiments of Guthrie (1968) and Barnett et al. (1971). This is important, because by the time malnutrition was induced, the critical period during which gross deficits in brain composition can be obtained was already ended. In the studies quoted, the activity was recorded during one hour periods in a running wheel (Guthrie, 1968) and during many days in a plus-maze like ours (Barnett et al., 1971). This is not at all irrelevant, because such results were not obtained in 10 minute sessions in an "open field" test (Guthrie, 1968; Franková & Barnes, 1968). Barnett and his coworkers (1971) found the hyperactivity to be much greater in rats that had received a low-protein diet than in rats that had received restricted amounts of a balanced diet. Consequently they speculated that the ratio between protein and carbohydrate might have been decisive in determining adult activity levels, which hypothesis is supported by findings of Barnes et al. (1968) and Lát (1965) who reported elevated

excitability in rats previously fed low protein or high carbohydrate diets. Both hypo- and hyperthyroidism have recently been reported to affect protein synthesis (see table 1) and thus may cause such protein/carbohydrate imbalance. The stimulatory effect of thyroid hormone on metabolic rate also point at this possibility. The same can be said with regard to the findings on learning ability: chemical as well as structural abnormalities may be the cause (Eayrs, 1968, 1971). Analysing behavioural results in this way, however, will not lead to anything but good correlations: treatments will cause both structural and behavioural changes, a causal relationship between them can not be proved along these lines.

The fact that neonatal hypo- and hyperthyroidism have different and generally even opposite effects on metabolism and structural development of the brain suggest that the identical effects on locomotor activity might be mediated by different mechanisms rather than by one and the same. Not even the behavioural basis of the hyperactivity is necessarily identical in the two groups (considering the two T3-treated groups B and C as one here). In analysing locomotor activity, Barnett (1958) describes two components of this behavioural measure: "appetitive" or "searching" behaviour, and exploration. Both these components might be affected by one or both of our kinds of treatment. As for the "appetitive" component, the adult hyperactivity after dysthyroidism and malnourishment may have reflected an increased "hunger drive" or search for food. In that case one should expect that the surplus of excursions would be made in the food arm of the maze. The results of this study, however, show clearly that the animals of all groups spread their visits rather evenly among all four arms; the least significant difference was even obtained on the data of the food arm in the A-group. As Barnett and his associates obtained essentially similar results after malnourishment, the conclusion must be that this concept of elevated "hunger drive level" fails as an explanation of the hyperactivity.

Barnett et al. (1971), in discussing the nature of the hyperactivity, mentioned three possibilities: (1) lower general threshold of arousal; (2) elevated responsiveness to novel stimuli; (3) slower habituation to environmental stimuli.

The results obtained in the present study on activity levels provide by themselves no conclusive evidence in favour of either of these three possibilities. When we consider these data in connection with those on learning ability in the MTU-treated rats, however, some indications emerge that slower habituation may be at least one of the components affected in this experimental group. When we look at the error scores in some more detail and divide the total test in three groups of four successive problems, we find that the MTU-treated rats made 53,9% more errors than their controls on problems 1-4, +35,1% on problems 5-8 and +30,0% on problems 9-12. This suggests that the animals needed the experience of more problems for getting used to the test situation and to the type of problems offered. When we compare the errors made on the first four

runs of each problem with those made on runs 5-8, we find that these experimental rats made 37,4% more errors on runs 1-4 and 47,5% more on runs 5-8. This means that the animals learn each problem more slowly than normal. These data give some indication that the rats might habituate more slowly than normal to environmental stimuli, which could have been a factor in the hyperactivity they showed.

Conversely the hyperactivity might have been a factor in the higher error scores of the MTU-treated rats. The fact that the T3-treated rats, at least as hyperactive as the thiouracil-treated ones, made not significantly more errors in the present study than their controls, suggests, however, that this is not the case, unless the age factor, (error scores were obtained two months later than activity recordings), would have been a crucial one for both measures. To solve the problems outlined here, much more research will have to be done on learning ability and locomotor activity simultaneously. More information on the role of habituation to the maze in the activity recordings could be obtained by registration from the day of placement in the maze onwards, and analysis of possible differences in the rate of activity-decrease. On the contrary, leaving the animals in the plus-maze permanently from weaning onwards would eliminate this habituation and create the opportunity to evaluate other factors, especially the age-factor. The registration of activity and error scores during longer periods as outlined above could reveal possible relationships between the two.

GENERAL DISCUSSION

In this chapter three items will have to be discussed:
1/ The nature of the treatments: the validity of the assumption that the treatments used in this study caused temporary hypo- and hyperthyroidism during the neonatal period.
2/ A classification of the effects.
3/ A comparison of the effects of the three treatments and those of malnutrition.

The nature of the treatments

For establishing that the treatments used in the present study may be held to have caused temporary hypo- and hyperthyroidism two requirements have to be met:
a/ the treatments must have been adequate in causing a hypo- or hyperthyroid state within a reasonably short period of time;
b/ the deviations from the euthyroid state must have been temporary and not present any longer at the ages at which measurements were taken.
ad a/ thiouracil derivatives are known to be effective within hours and even to lose their maximal effectiveness within hours after administration (Astwood, 1965); the dosage used in this study was more than adequate. The dosage of T3 given to the B-animals was several times higher than the daily amount required to maintain euthyroid state in athyroid individuals (Barker, 1964).
Ad b/ the results of the present study on thyroxine levels described in the chapter on physical development give no reason to suspect that significant deviations from the euthyroid state had lasted into adulthood in any of the treatment groups.

The conclusion seems justified that all adult abnormalities classified below are indeed caused by temporary deviations from the euthyroid state in infancy.

Classification of the effects

That the adult abnormalities observed in this study were caused by devia-

tions from the euthyroid state in early infancy does not necessarily mean that all these effects were already present at the time of treatment, nor that all abnormalities induced immediately at the time of treatment persisted into adult life. In fact thyroid deficiency or excess may exert a diversity of effects, that are not all independent of each other, but by a network of interactions either have causal relationships or neutralize one another. The results obtained in this work will be discussed in terms of the following classification:

(1) *immediate effects* appearing immediately or shortly after the onset of treatment and disappearing immediately or shortly after the termination of the treatment;
(2) *temporary short-term effects* appearing during treatment, but not present any more in the adult animal;
(3) *persistent short-term effects* appearing during treatment and still present in the adult animal;
(4) *long term effects* appearing not earlier than some time after termination of the treatment.

Immediate effects

To this category belong effects on transcriptional and metabolic processes; probably all other effects are secondary to these. Similar effects can probably be obtained by malnutrition, since malnutrition has many secondary effects in common with our treatments. This will be discussed in more detail below.

Temporary short-term effects

As discussed in the chapter on physical development, the possible impairment of pituitary function in hypothyroid animals responsible for the temporary stunting of growth in this A-group, did not last into adult life. Consequently both this endocrine effect and the growth retardation belong to this category of effects.

The most interesting temporary effects of deviations from the euthyroid state in either direction were the changes in timing of maturational processes in the nervous system and the skeleton. These effects may seem ultimately less important than some others, because the animals will eventually end up completely mature, be it some time later than normal after thiouracil treatment and earlier after T3 treatment. As discussed for skeletal maturation in the chapter on physical developments, however, such changed timing of maturational events may be decisive for the development of other measures. Schapiro (1968) and Hamburgh (1968) have argued that since cell proliferation and cell differentiation are mutually exclusive events, the altered timing of differentiation induced by abnormal thyroid hormone levels will affect the length of the period during which cell proliferation can take place. This explains how treatment with thyroid hormones, by inducing differentiation of tissues before normal numbers of cells

have been formed, will cause a persistent decrease in cell number of the brain as well as of the body as a whole, whereas hypothyroidism does not affect cell number, but only cell size by its effects on protein synthesis.

Another effect of a transient nature was the lower level of locomotor activity of the MTU-treated rats at one month of age.

Persistent short-term effects

The irreversibility of the stunted growth in the T3-treated rats assigns it to this group of effects. The growth pattern of the C-group, however, requires some reservation to be made. As mentioned in the discussion on growth, it might be necessary to distinguish two different effects on growth in these animals: (1) the effect seen during the first few weeks, a temporary short-term effect, probably mediated by metabolic disturbances; (2) the effect seen several weeks later, a long-term effect, mediated by the advancement of skeletal maturation.

Less doubt in this respect exists about the effects on brain development found at autopsy: the lower brain weights were probably present already at an early age (table 1).

Long-term effects

Clearly the hyperactivity of all experimental animals belongs to this category, since it was not yet present at one month. Less certainty exists about the impairment of learning ability, because this has not been measured very early in life.

The remarkable abnormalities of testis weight probably are long-term effects too, since the results of the complementary study indicate that they do not emerge before puberty.

Thyroid and nutrition

For analysing the mechanisms underlying the effects classified above, it may be useful to compare these effects with those obtained by early nutritional deprivation, because the many similarities between the consequences of dysthyroidism and malnutrition suggest some relationship.

Food restriction throughout the suckling period causes a persistent impairment of growth in weight, not repaired by ad libitum feeding after weaning (Widdowson & McCance, 1960). Brain and other organs are smaller than normal, due to reduced number of cells (Winick & Noble, 1966; Guthrie & Brown, 1968). Animals rehabilitated from day 10 after malnutrition for 9 days will show no such persistent deficits in the brain (Winick et al., 1968). The emergence of reflexes and several other maturational criteria are delayed in malnourished rats (Eayrs & Lishman, 1955; Smart & Dobbing, 1971). Malnourishment in infancy may also affect adult behaviour; hyperactivity was already discussed in the previous chapter. The stunting of adult body size by postnatal

malnutrition is not necessarily accompanied by changes in behavioural development; conversely, however, behavioural abnormalities caused by nutritional deprivation in infancy are always accompanied by stunting of body size (Barnes et al., 1968). Impairment of learning ability is reported by Eayrs & Lishman (1955), who underfed their rat pups by restricting the time spent with their mother. As this technique deprives the pups from maternal care as well, their results may be attributable to social deprivation. This view is supported by the absence of differences in learning ability when other techniques are employed, such as underfeeding the mother (Franková & Barnes, 1968; Guthrie, 1968) or keeping the pups with non-lactating foster mothers displaying normal maternal behaviour (Slob et al., subm.).

Comparing the effects of early nutritional deprivation summarized above with the effects of early hypo- and hyperthyroidism reported in the present paper and by other authors, it becomes clear that the hypothesis that the thyroid exerts its influence on development merely in an indirect way by its influence on the intake and utilization of nutrients, is far too simple. For, the only effect that all three treatments have in common, is the hyperactivity. The lasting structural deficits obtained by early nutritional deprivation are quite similar to those seen after neonatal treatment with thyroid hormones, whereas the retardation of maturational processes is conform to that seen after hypothyroidism. The stunting of adult body size after early food deprivation in spite of delayed maturation suggests that these two phenomena are both due to a shortage of all kinds of nutrients that are required for mitosis as well as for differentiation. The accelerated maturation in the T3-treated rats on the contrary provides evidence that this latter treatment is more selective in its effects on chemical processes concomitant with development.

It therefore seems justified to conclude that, whereas the effects of malnourishment are caused by a general shortage of nutrient materials at the cellular level, the effects of deviations from the euthyroid state are mediated by selective acting upon intracellular thyroid-dependent or thyroid-sensitive regulatory mechanisms.

SUMMARY

1. In order to discriminate between temporary and persistent effects of transient deviations from the euthyroid state during the neonatal period on physical and behavioural development, newborn litters of 8 rats were assigned to one of three experimental groups. Half of the males from each litter received daily injections with:
   A. MTU (methylthiouracil) in Na-alginate solution; 2 mg on day 2-8, 4 mg on day 9-15.
   B. T3 (L-triiodothyronine) in 50% propyleneglycol; 0,5 μg on day 2-15.
   C. T3 in 50% propyleneglycol; 40-50 μg on day 2-4.
   The other males in the litter received equal amounts of solvent only.
2. Body growth was studied by weekly determination of body length and weight. Weekly X-rays were made and skeletal maturity scores rated from them.
   MTU-treated rats showed a considerable growth retardation during some weeks initially, but growed faster than controls later, ultimately reaching normal adult body size. Their skeletal maturation was also retarded. Their body length appeared to be at all ages about normal for their skeletal age.
   T3-treated rats of both groups showed a persistently lower growth rate than normal, their skeletal maturation was accelerated. Their body length was at all ages far below normal for their skeletal age.
   The relations between skeletal maturation and growth are discussed.
3. The effects of the treatments on the endocrine system were studied by determination of serum thyroxine levels before and after a one week stay in a cold room. After that autopsy data were collected on the weight of brain, thyroid, adrenals, testes and seminal vesicles.
   No significant differences were found in serum thyroxine levels. The MTU-treated rats had a slightly heavier thyroid gland and much heavier testes than normal, whereas their brain weight was too low. T3-treated rats had smaller testes than normal only in the B-group; in both groups the brain weight was affected less than body weight. Weight of adrenals and thyroid was normal

for body weight.
In none of the experimental groups differences were found in the weights of the seminal vesicles.
Mechanisms by which the obtained differences could have brought about are discussed.

4. Nervous system maturation was studied by determining the day the eyes opened and righting reflex and placing reaction first appeared. These criteria for maturation were delayed in the A-group and advanced in the B- and C-group.
Locomotor activity was recorded during one week when the animals were almost one month old and again two months later. At one month the A-group was less active than normal, the T3-treated animals showed no difference with controls. Two months later all three groups showed considerable hyperactivity. The structural and behavioural backgrounds of this phenomenon are discussed in connection with the findings on learning ability.
Learning ability was tested in a Hebb-Williams maze when the animals were between 4 and 6 months old. Only the MTU-treated rats made significantly more errors than control animals.
5. The nature of the treatments and the interrelationships of the obtained effects are discussed, a classification of these effects according to time of appearance and persistence is given and finally a comparison is made between the effects of dysthyroidism and those of malnutrition.

SAMENVATTING

1. Met het doel onderscheid te maken tussen voorbijgaande en blijvende effekten van tijdelijke afwijkingen van de normale schildklierfunktie kort na de geboorte op de ontwikkeling van lichaam en gedrag, werden pasgeboren nesten van 8 ratten ingedeeld bij een van 3 experimentele groepen. De helft van de mannetjes uit elk nest ontving dagelijks injecties met:
   A. MTU (methylthiouracil) in Na-alginaatoplossing; 2 mg op dag 2-8, 4 mg op dag 9-15.
   B. T3 (L-triiodothyronine) in 50% propyleenglycol; 0,5 μg op dag 2-15.
   C. T3 in 50% propyleenglycol; 40-50 μg op dag 2-4.

   De andere mannetjes in het nest ontvingen gelijke volumes van het oplosmiddel alleen.
2. Lichaamsgroei werd bestudeerd door wekelijkse bepaling van lichaamslengte en -gewicht. Wekelijkse röntgenopnamen werden gemaakt ter bepaling van skeletrijpingsscores.

   De met MTU behandelde ratten vertoonden een aanzienlijke groeiremming gedurende enkele weken in het begin, maar groeiden naderhand sneller dan controledieren om uiteindelijk normale volwassen afmetingen te bereiken. Hun skeletrijping was vertraagd. Op alle leeftijden bleek hun lichaamslengte ongeveer normaal te zijn voor hun skeletleeftijd.

   De met T3 behandelde ratten vertoonden een permanent lagere groeisnelheid dan normaal, hun skeletrijping was versneld. Hun lengte was doorlopend ver beneden normaal voor hun skeletleeftijd.

   De relaties tussen skeletrijping en groei worden besproken.
3. Effekten der verschillende behandelingen op het endokriene systeem werden bestudeerd door serum thyroxine concentraties te bepalen voor en na een zevendaags verblijf in een koude kamer. Daarna werd autopsie verricht ter bepaling van de gewichten van hersenen, schildklier, bijnieren, testikels en zaadblazen.

   Er werden geen significante verschillen in thyroxineconcentraties gevonden. De met MTU behandelde dieren hadden een iets zwaardere schildklier en veel

zwaardere testikels dan normaal, hun hersengewicht was iets te laag.
De met T3 behandelde dieren hadden een te laag testikelgewicht, echter alleen in de B-groep; in beide groepen was het hersengewicht verlaagd, doch in mindere mate dan het lichaamsgewicht. Het gewicht van bijnieren en schildklier was normaal ten opzichte van het lichaamsgewicht.
In geen van de groepen werden verschillen in zaadblaasgewicht gevonden.
De mechanismen worden besproken waardoor de gevonden verschillen ontstaan zouden kunnen zijn.

4. De rijping van het zenuwstelsel werd onderzocht aan de hand van de dag waarop de ogen opengingen en de "righting reflex" en de "placing reaction" voor het eerst aanwezig waren. Deze rijpingscriteria bleken verlaat in de A-groep en vervroegd in de B- en C-groep.
Lichamelijke aktiviteit werd bepaald gedurende een week toen de dieren bijna een maand oud waren en nogmaals twee maanden later. Een maand oud waren de A-dieren minder aktief dan normaal, de B- en C-dieren vertoonden geen verschil met controledieren. Twee maanden later vertoonden alle drie de groepen aanzienlijk grotere aktiviteit dan normaal. De mogelijke achtergronden van deze hyperaktiviteit op het gebied van structuur en gedrag worden besproken in samenhang met de resultaten van het onderzoek naar leervermogen.
Dit leervermogen werd getest met behulp van een "Hebb-Williams maze" toen de dieren tussen 4 en 6 maanden oud waren. Alleen de met MTU behandelde ratten maakten duidelijk meer fouten dan hun controledieren.
5. De aard der toegepaste behandelingen en de onderlinge samenhang van de gevonden effekten wordt besproken, er wordt een indeling van deze effekten gegeven op grond van de tijd van verschijnen en de blijvendheid en tenslotte worden deze effekten vergeleken met die van neonatale ondervoeding.

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