# PERINATAL ENDOCRINE AND NUTRITIONAL FACTORS CONTROLLING PHYSICAL AND BEHAVIOURAL DEVELOPMENT IN THE RAT

A. Koos Slob

to all who make
living worthwhile

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PROEFSCHRIFT

ter verkrijging van de graad van
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te 16.00 uur precies door

ADRIAAN SLOB

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Offset drukkerij bureau Vereniging voor Christelijk Voortgezet Onderwijs
te Vlaardingen

Promotor: Prof. Dr. J.J. van der Werff ten Bosch

Co-referenten: Prof. Dr. M.W. van Hof

Prof. Dr. H.K.A. Visser

*"..... WHAT IS PERCEIVED DEPENDS NOT ONLY ON WHAT IS BEING LOOKED AT BUT ON THE STATE OF THE PERCEIVER."*

M.L. Johnson Abercrombie. *The anatomy of Judgement*, page 27. Penguin Books 1969.

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CONTENTS

# GENERAL INTRODUCTION

Normal *prenatal* growth and development of mammals is brought about by various extremely complex factors of genetic, nutritional, and hormonal nature. The interaction of all these factors leads to the establishment of normal structures and physiological and psychological functions in the newborn and thus to the possibility of an adequate development after birth.

Normal *postnatal* growth and development also depend on various endogenous and exogenous phenomena such as hormones, social environment, and nutrition.

In the last decades numerous investigations have been undertaken to assess the contribution of these various factors before and after birth. In general we may state that growth and development depend on "organizing processes" (Scott, 1962). In other words: "what has been organized during individual development can be reorganized only with difficulty, as organization inhibits reorganization. The organizational process can be influenced only at the time it is occurring, that is, during the critical developmental period" (Křeček, 1971, page 233). This is clearly illustrated by the organizing effects of gonadal hormones on neural tissues mediating specific male and female behaviours. In some species the organizing (or critical) period occurs after birth whilst in others this occurs before birth. Removal of the gonads and administration of gonadal hormones in *newborn* rats and hamsters cause endocrine and behavioural disturbances which manifest themselves after long latent periods, sometimes only after sexual maturation, and very often result in a complete loss of reproductive functions, including mating behaviour (Barraclough, 1961; Barraclough and Gorski, 1962; Swanson and Van der Werff ten Bosch, 1963; Harris and Levine, 1965; Swanson, 1970, 1971).

Administration of androgenic hormones during *prenatal* organizing periods gives rise to masculinization of the external genitalia at birth and changes in adult behaviour in the female guinea pig (Phoenix, Goy, Gerall and Young, 1959), ferret (Baum, 1972), dog (Beach and Kuehn, 1970), and rhesus monkey (Phoenix, Goy and Young, 1967).

Although much work has been done on the organizing effects of gonadal (and other) hormones on behaviour, little is known of the organizing effects of these hormones on somatic growth and development. Since there

is a marked sex difference in body size in most mammals, the question arises whether part or all of this difference is caused by an early influence of gonadal hormones on mechanisms which control body growth.

In the first part of the present study an attempt has been made to establish the role of the gonads and of androgens during late prenatal and early postnatal life in the control of subsequent somatic growth and development in the white laboratory rat.

Non-endocrine factors such as manipulation of the young, the number of young in a litter and the mode of rearing (e.g. in isolation) can also influence the further development of an individual if they are operative during a critical period. Many experiments have shown that handling of the newborn does not only alter the development of its behaviour, but also permanently influences endocrine mechanisms (Denenberg, 1968; Levine and Mullins, 1968). Rats reared in large litters (of 12 animals) displayed behaviours in adulthood which differed from those of animals reared in litters of 6 (Seitz, 1954). Isolation (the lack of sufficient social interaction) during early development has also been shown to affect adult behaviour in the rat (Nováková, 1966; Gruendel and Arnold, 1969), the guinea pig (Valenstein and Young, 1955), the dog (Scott, 1968), the sheep and the goat (Moore, 1968), and in the rhesus monkey and the chimpanzee (Mason, Davenport and Menzel, 1968).

Also a great number of investigations have been performed on the effects of undernutrition and/or malnutrition early in life on subsequent growth and development and on behaviour in adulthood (e.g. Altman, Das and Sudarshan, 1970). Most of these investigations, both in the human and in laboratory animals, suggest that physical and mental development become permanently affected. However, if these studies are scrutinized more closely, there appears to exist no solid evidence to confirm this view. In the human studies it is impossible to differentiate between effects of malnutrition and of maternal, social and educational deprivation. Animal studies have likewise failed to provide good evidence, since undernutrition and malnutrition have always been confounded with other variables such as isolation (maternal and littermate deprivation), variations in litter size, and handling.

In the second part of the present study, also in the rat, the effects have been studied of neonatal food deprivation with or without maternal deprivation on growth and development, and on adult behaviour and learning ability.

This work thus consists of two parts:

A. growth and development of male and female rats following various prenatal, neonatal, and late postnatal endocrine treatments;

B. growth and development of male and female rats following various regimens of neonatal food deprivation.

Part of this work, performed in collaboration with Catherine E. Snow and Els de Natris-Mathot, has been accepted for publication by the journal: "Developmental Psychobiology". Two abstracts will be published in: "Acta paediatrica Scandinavica" and "Brain Research".

# GENERAL METHODS

## Animals and treatments

All rats used in these experiments came from a Wistar strain, Amsterdam R-stock. The breeding schedule was such that several litters were born on the same day. Except when stated otherwise, several litters were mixed on the day of birth ( ay 1) and randomly redistributed in such a way that each litter contained 8 pups. On ay 25 the young were weaned and placed in groups of 3 or 4 animals to a cage. From this age on all animals had free access to water and standard laboratory rat chow, except when temporarily food deprived for specific tests. Temperature in the animal quarters averaged 22°C, and lighting was artificial only: lights were on for 14 hours per day during the first four experiments (A) and 12 hours per day during the last three experiments (B).

Operations within 24 hours of birth were carried out under ice anaesthesia, at later ages under ether anaesthesia.
Ovaries were removed through bilateral dorsal incisions, testes (with epididymides) were removed through a ventral caudal incision.

Androgen administration consisted of subcutaneous injections of testosterone propionate (TP) in the neck area. Specifications of the dosage and the injection schedule will be presented with the relevant experiments.

## Measurement of physical development

Length of head-plus-trunk and tail length were measured in mm using a "Western Reserve Measuring Board" described by Acheson, McIntyre and Oldham (1959), and will be referred to as body length and tail length respectively. Body weights were obtained on a Berkel balance to the nearest gram.

For taking X-rays 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 one meter. From these radiographs scores for skeletal maturity were obtained using the rating technique of Hughes and Tanner (1970). The bones used in these studies were (maximal score in parentheses): humerus (14), radius (14), ulna (15), metacarpals (13) and proximal phalanges (11). The maximal total score an animal could reach in this way was 67. Furthermore, the length of the radius was measured.

## Behavioural measures

In adulthood (sub)groups of animals of the neonatal food deprivation experiments (B) were subjected to a number of tests (see Table 1).

| Test | A measure of: | Neonatal food-deprivation regimen | | |
|---|---|---|---|---|
| | | Foster mother | Incubator | Big litter |
| Open field | Exploratory behaviour Emotionality | 75 | 90 | 90 |
| Elevated Runway | Motor ability | 80 | | |
| Residential Plus Maze | Baseline locomotor activity | 110-150 | 200-230 | 210-240 |
| Hebb-Williams Maze | Food motivated learning ability | 130-170 | 120-150 | 140-175 |
| Thompson Box | Shock motivated learning ability | 230-245 | 240-260 | |
| Pup retrieval | Maternal behaviour | 250-270 | | |
| Mating | Sex behaviour | 300 | | |

*Table 1. Summary of the different tests used in the neonatal food deprivation experiments. Numbers indicate ages (in days) at which the animals were tested.*

*Exploratory behaviour and emotionality*

The test chosen for measuring exploratory behaviour and emotionality

was the open field. The animals were individually placed in the centre of a circular open field (Figure 1), 77 centimeters in diameter, and observed for 10 minutes by one or two experimenters. During this test period ambulation (number of squares entered) and defaecation (number of boluses produced) were counted.

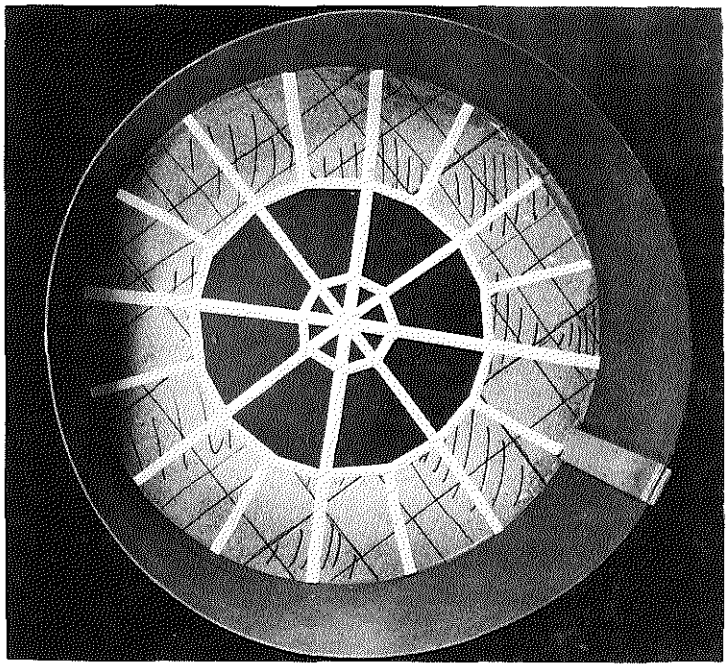


*Figure 1. The open field apparatus used in testing exploratory behaviour and emotionality.*

*Baseline locomotor activity*

A residential plus-shaped maze (Barnett, Smart and Widdowson, 1971; Pelt, 1972) consisting of a central nest box with arms 40 centimeters long extending from it was used to study baseline locomotor activity. A pair of photoelectric cells in each arm were triggered by the animal whenever it left or entered the nest box, i.e. producing an automatic record of each excursion from the nest box, the time of the excursion, the time of the return to the nest box, and the arm which was entered.

One arm contained food, one contained water, and two were left empty.

The apparatus was slightly modified after it had been used in the foster mother food deprivation experiment. The modifications were: the metal nest box top was replaced by a perspex one, and the food bin was modified to allow measuring of the daily food intake (see Figures 2, 3 and 4).

*Figure 2. The testroom with the twelve residential plus mazes used in recording baseline locomotor activity. Note that each maze is provided with its own light source, a 20 Watt fluorescent tube placed diagonally above it.*

The animals were placed in the plus maze for periods of eight or nine days. Four days were allowed for the animals to adapt to the new living conditions and the slightly different light schedule (the latter only in the foster mother food deprivation experiment); activity on these days was not scored. In order to avoid any bias by disturbances during the day only records from the dark period were included in the

calculations.

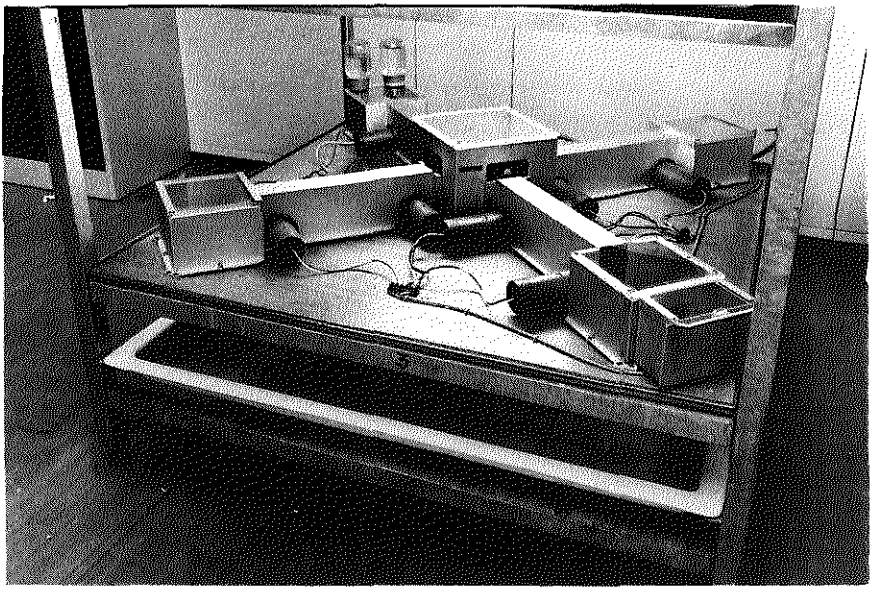


*Figure 3. The residential plus maze for measuring baseline locomotor activity.*

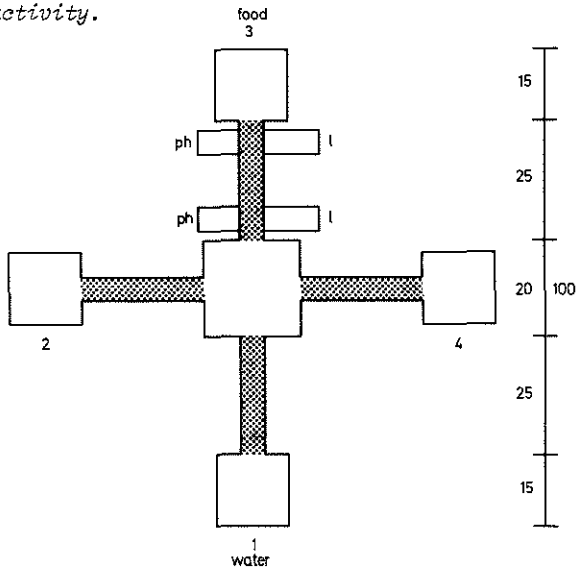



*Figure 4. Diagram of the residential plus maze. Photoelectric cells and infrared light sources (ph and l) were present in each arm. Scale is given in centimeters.*

*Food motivated learning ability*

The test chosen for measuring learning ability was the "closed field test" first described by Hebb and 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 experiments were almost identical to those described by Rabinovitch and Rosvold (1951).

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 outlined in black (Figure 5). The maze has a grid top and two boxes,

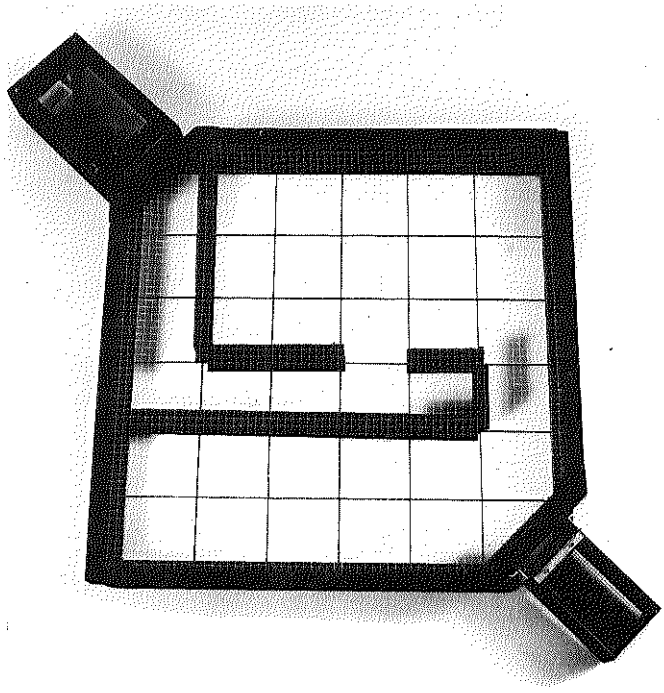


*Figure 5. The Hebb-Williams maze used in testing food motivated learning ability.*

which can be attached to two opposite corners of the maze. The boxes are provided with a tip-up transparant lid and a guillotine-door giving access to the maze and are used as start and goalbox respectively. With

the maze are a number of black wooden barriers and 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 and Rosvold are illustrated in figure 6.

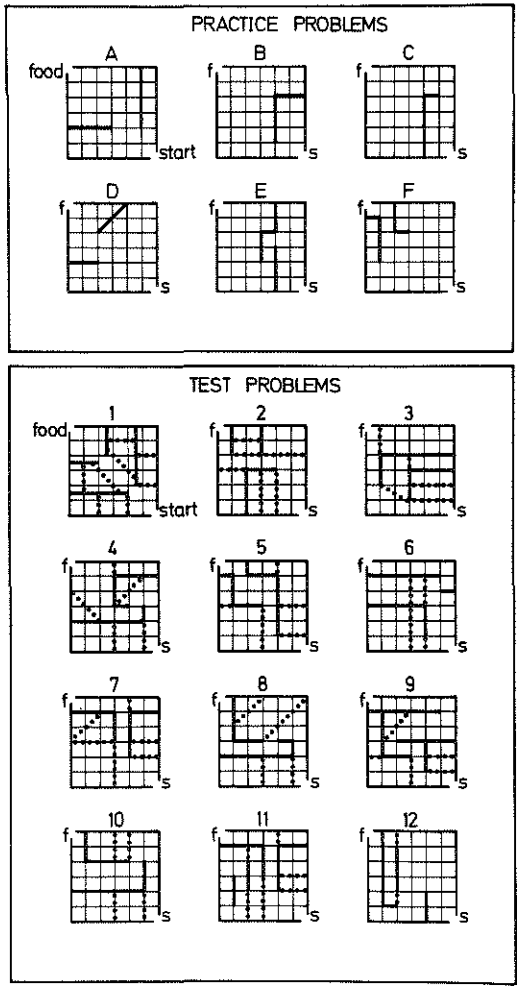
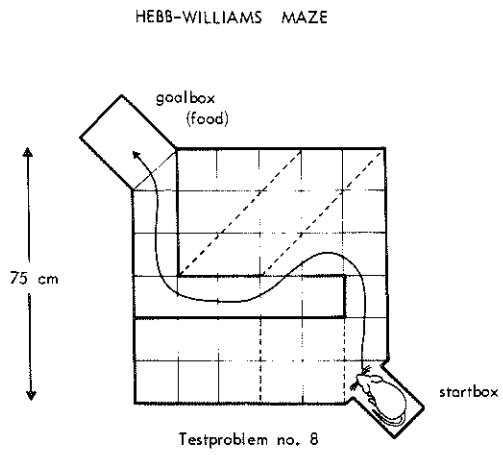



*Figure 6. The various problems of the Hebb-Williams maze. For explanation see text. (After Rabinovitch and Rosvold, 1951)*

The animals were food-restricted during the entire procedure

described below, getting food for only one period per day, in the goal-box between runs and in special cages immediately after completing their runs for the day.




Procedure:

| | | |
|---|---|---|
| A. adaptation | 2 days | |
| B. training | 3 days | 6 practice problems; time scored<br>criterion: 9 consec. runs within 60 sec. |
| C. testing | 4 days | 12 test problems; errors and time scored |

*Figure 7. Diagram of the Hebb-Williams maze with one test problem shown by solid lines. For a more detailed description of the testing procedure see text.*

The procedure itself comprised three stages (see Figure 7).

1. An adaptation period of two days, during which the animals were left in the maze in small groups for two to three hours per day with food in the goalbox in order to get accustomed to the maze and the location of the food.
2. A training period, during which the animals had to run the practice problems A - F. This was done individually, two problems per day, until all animals had reached the criterion for beginning the real testing. The criterion was the completion of nine runs on the same

problem within a total of 60 seconds. For the six problems together this took about three to four days.

3. The testing period of four days. Each day the animals had to run three test problems eight times in succession. Time was scored and the number of errors was counted. Each time when an animal entered an error zone (in Figure 6 demarcated with dotted lines) with both forepaws this was counted as an error. The total number of errors made on 12 x 8 runs was used as measure in these experiments.

*Shock motivated learning ability*

Since learning in the Hebb-Williams maze is motivated with food reward, it was thought desirable to include another test of learning.

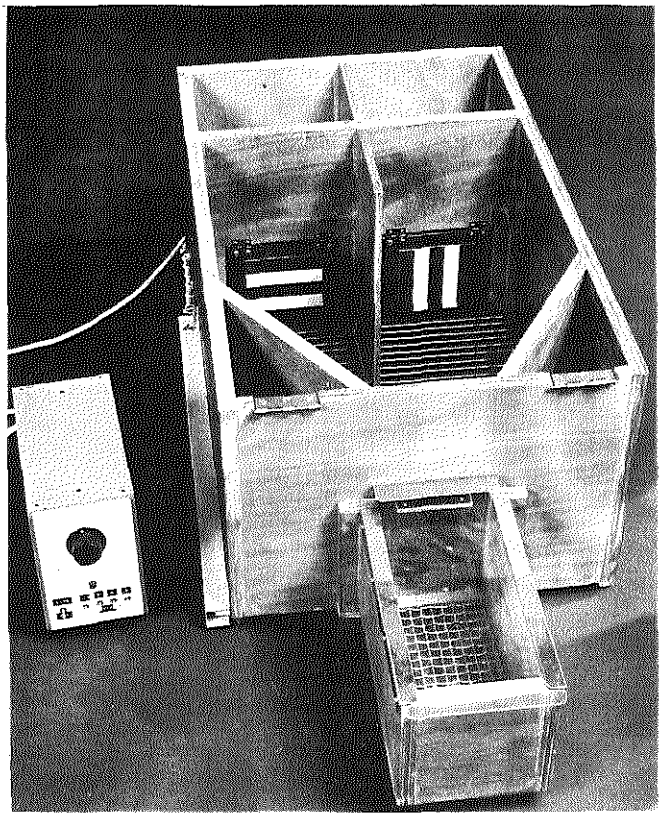


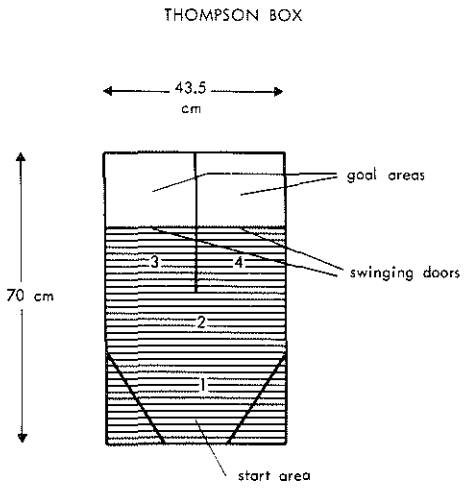
*Figure 8. The Thompson box used in testing shock motivated learning ability.*

Accordingly, animals were taught one or two visual discrimination problems in the shock-motivated Thompson box (Thompson and Bryant, 1955; Buchtel, 1970).

The apparatus measured 70 x 43.5 cm, 30 cm high (Figure 8). It was constructed of varnished plywood except for the grid area (.3 mm stainless steel rods spaced 1.5 cm apart). The discrimination stimuli consisted of: (a) a black versus white square; and (b) vertical versus horizontal blue stripes on a white background. The stimulus cards were centered on 10 x 11 cm swinging doors with their bottom edges 1 cm above the grid. The grid areas 1, 2, 3 and 4 were independently wired so that they could be individually electrified.

The procedure comprised two stages (Figure 9).

1. A training period of one or two days. The animal was placed in Area 1 facing the goal area (no visual cues) and was given brief shocks




Procedure:

| | | | |
|---|---|---|---|
| A. | training | 1 or 2 days | criterion: 10 "negative runs" within 100 sec. |
| B. | testing | 4 to 8 days | 20 or 40 runs per day; criterion: 18 correct choices out of 20 runs |

*Figure 9. Diagram of the Thompson box. For a more detailed description of the testing procedure see text.*

if it did not leave Area 1 within 5 seconds or Area 2 within 15 seconds. After the rat had entered one of the goal areas, it was left there for a 20 - 30 second interval; such an interval was allowed following each run. After the subject made a run to the goal area without any shocks, the entrance chosen was blocked and the shock turned on in front of it. After the animal ran through the unblocked opening without any shocks, that door was locked, the other one unlocked, and the shock shifted to the new side.
The training was complete when the animal had entered the goal area following ten wrong choices ("negative runs") within a total of 100 seconds.

2. The testing period of 4 to 8 days during which discrimination learning began. Metal-backed stimulus card holders were held by magnets imbedded in the doors and the position of the positive stimulus was changed according to a random table (a modified series after Gellermann, 1933) during the intertrial interval. On trials when the discriminanda were not shifted, the experimenter went through the usual motions of changing them in order not to give the animal any unintentional cues about the position of the positive stimulus on the following trial.
Animals received 20 or 40 trials each day until they reached "the criterion" of 18 correct choices out of any 20 consecutive runs. The numbers of correct and incorrect choices and the time taken to run from Area 1 to the goal area were recorded.

*Further behaviour tests*

Specifications of the remaining behavioural tests will be presented with the relevant experiments.

## Autopsy data

After anaesthetizing the animals with ether about 4 - 7 ml of blood was collected from the orbit for thyroid hormone assays. The animals were subsequently killed with chloroform, and body weight, body length and tail length were measured in the same way as described before. Various organs were removed and weighed on a Mettler balance to the nearest mg.

## Thyroid hormone assays

Blood serum thyroxine and triiodothyronine levels were determined in the laboratory of Dr.G. Hennemann and Ir. R. Docter at the Department of Internal Medicine III of the Rotterdam Medical Faculty.

Thyroxine concentrations (values corrected for extraction) were measured through a competitive protein binding assay (Abbott Laboratories).

Triiodothyronine concentrations were measured directly in the serum through a radioimmunoassay method (Docter and Hennemann, pers. comm.1972).

## Statistical methods

Most data were subjected to the Mann-Whitney *U* test and Student's *t* test. The only exceptions were the somatic growth data of the foster mother food deprivation experiment; these data were subjected to a Two-way Analysis of Variance.

Where median values are presented the data were subjected to the Median test and the probability of the observed values determined by either the Fisher test or the $X^2$ test (Siegel, 1956).

Unless otherwise indicated, two-tailed tests of significance were used and the .05 level of probability adopted as the level of statistical significance.

# A. GROWTH AND DEVELOPMENT OF MALE AND FEMALE RATS FOLLOWING VARIOUS PRENATAL, NEONATAL, AND LATE POSTNATAL ENDOCRINE TREATMENTS

## INTRODUCTION

One of the most obvious expressions of sexual dimorphism in many mammalian species is the difference in adult body size between males and females. The adult male is usually heavier and larger than the adult female (e.g. Tanner, 1962; Wade, 1972). This difference in body size is already noticeable at birth, most obviously in species that are born in a relative mature state such as the guinea pig (Goy, Phoenix and Meidinger, 1967; Slob, Goy and Van der Werff ten Bosch, 1972), but also in species immature at birth such as the rat (King, 1915). This indicates that prenatal factors of genetic, hormonal and/or nutritional origin are involved. For the guinea pig it has been shown that prenatal exogenous androgen (from Day 24 through 41 of foetal life) affects postnatal growth of the genetic female in such a way that the prepuberal growth pattern becomes similar to that of the male (Slob, Goy and Van der Werff ten Bosch, 1972). In the rat gross changes in the body growth patterns occur both after gonadectomy (Stotsenburg, 1913; Moore, 1919; Van Wagenen, 1928; Freudenberger and Billeter, 1935; Holt, Keeton and Vennesland, 1936; Freudenberger and Hashimoto, 1938, 1939; Rubinstein, Abarbanel and Kurland, 1939) and following the administration of sex hormones after birth (Rubinstein and Solomon, 1940, 1941; Swanson and Van der Werff ten Bosch, 1963; Smith and Allison, 1965; Valenstein, 1968). In both these species, therefore, sex hormones may play a significant role in the causation of the normal sex differences in growth patterns.

Some recent studies into the effects of gonadectomy of the rat very early in life have yielded equivocal results as to the magnitude of the contribution of sex hormones to the sex difference in adult body size. On the one hand (Grunt (1964a, b) found that removal of the gonads obliterated the sex differences in growth patterns (skeletal lengths and body weights); identical results were obtained after operations on the day of birth and on Day 25. Harvey, Hervey and Hutchinson (1972) reached the same conclusion, after gonadectomy on

Day 1. On the other hand Bell and Zucker (1971) reported that neonatal gonadectomy (males: at birth; females: on Day 4) did not abolish the sex differences in body weight growth: from 75 days of age onward castrated male rats weighed significantly more than ovariectomized females.

The purpose of the present study was to assess the contribution of androgens before and after birth, and of the gonads after birth, to sex differences in the pattern of growth. Therefore, 4 experiments were carried out in which body growth was always studied longitudinally, i.e. identical individuals were measured repeatedly.

In the first experiment (Experiment 1. Untreated male and female rats) the postnatal growth pattern of normal male and female rats was recorded. Also the relationship between the body weights at birth and in adulthood was studied. In the second experiment (Experiment 2. Postnatally gonadectomized male and female rats) the effects of gonadectomy at birth and on Day 21 on subsequent growth were studied. In adulthood the effects of prolonged androgen administration on body weight and male copulatory behaviour were studied in these animals. The third experiment (Experiment 3. Female pseudohermaphroditic rats) was designed to examine the effects of prenatal androgen administration, with or without prolonged neonatal androgen treatment, upon postnatal growth of intact and Day 1 ovariectomized female rats. Finally, in the fourth experiment (Experiment 4. Neonatally androgenized female rats) the role of the gonads in the growth of female rats was studied in animals that had received a single injection of a high dose of androgen on the third day of life. Table 2 summarizes the designs of the 4 experiments. All 4 experiments were carried out simultaneously.

The collaboration of Dick Elhorst and Frits J.M. Vels in collecting the data, and of Chris de Jong in analysing the radiographs is gratefully acknowledged.

| Experiment | Sex | N | Prenatal TP (days 16–20 of gestation) | Gonadectomy | | Postnatal TP on days | Whole body X-rays days 28, 56, 91 | Duration of longitudinal growth study (days) | Age (days) at tests for male sex behaviour | Age (days) at autopsy |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Day 1 | Day 21 | | | | | |
| Untreated | ♂ | 46 | | | | | | 126 | | |
| | ♀ | 46 | | | | | | | | |
| Postnatal gonadectomy | ♂ | 4 | | | | 115–210 | + | 210 | 176 and 210 | 350 |
| | | 4 | | | | | + | | | |
| | | 4 | | + | | 115–210 | + | | | |
| | | 7 | | + | | | + | | | |
| | | 7 | | | + | 115–210 | + | | | |
| | | 6 | | | + | | + | | | |
| | ♀ | 5 | | | | 115–210 | + | | 210 | |
| | | 4 | | | | | + | | | |
| | | 6 | | + | | 115–210 | + | | | |
| | | 5 | | + | | | + | | | |
| | | 5 | | | + | 115–210 | + | | | |
| | | 4 | | | + | | + | | | |
| Pseudohermaphrodites | ♀ | 5 | + | | | 2–20 | + | 126 | | 150 |
| | | 4 | + | | | | + | | | |
| | | 8 | + | + | | 2–20 | + | | | |
| | | 4 | + | + | | | + | | | |
| Neonatal androgenazation | ♀ | 13 | | + | | 3 | + | 126 | | 140 |
| | | 9 | | + | | | + | | | |
| | | 8 | | | | 3 | + | | | |
| | | 11 | | | | | + | | | 160 |

*Table 2. Summary of the different experiments showing the various prenatal, neonatal and late postnatal endocrine treatments and the data collected subsequently.*

# EXPERIMENT 1. UNTREATED MALE AND FEMALE RATS

## METHODS

### Procedure and subjects

Pups from 11 litters were used to constitute 10 litters in such a way that most of them were left with their own mother. On the day of birth (Day 1), within 3 to 4 hours following the onset of delivery, the pups were sexed, weighed, and marked with coloured ink. Litters were kept at a standard size of 10 pups; 8 young that died during the suckling period were replaced by animals of the same age, and left in the litter till the time of weaning.

### Measurement of physical development

The rats were weighed between 09.00 and 11.00 hours every 7th day until Day 126, with the exception of Days 112 and 119. The animals were remarked every other day until about Day 20 when they received a numbered eartag. Beginning on Day 30 females were checked daily for opening of the vagina; at the day of vaginal opening the animals were weighed.

## RESULTS

### Physical measures

*Somatic growth*

The longitudinal body growth data of 46 males and 46 females are shown in figure 10. The body weight curves for male and female rats diverged from Day 28 on, thus showing the well-known sex difference in postnatal growth (e.g. Swanson and Van der Werff ten Bosch, 1963). The peak in body weight velocity occurred in the 6th week for the female rats and one week later for the males. There was a secondary peak in

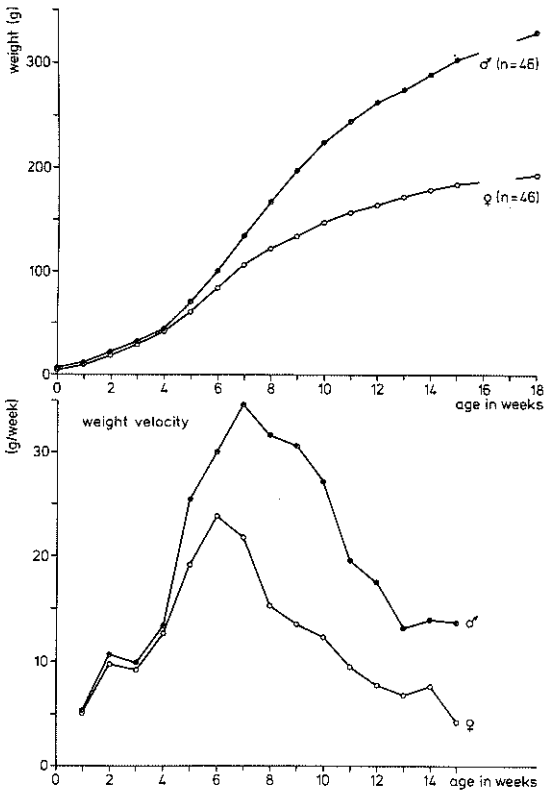



*Figure 10. Untreated male and female rats. Growth in body weight.*

the growth rate of female rats, or a halt in the deceleration in the growth velocity in the 14th week of life. The male growth curve showed the same phenomenon to a minor degree. This corroborates findings of Swanson and Van der Werff ten Bosch (1963) and Hughes and Tanner (1970) for the rat; and Slob, Goy and Van der Werff ten Bosch (1972) for the guinea pig.

*Sequelae of differences in body weight at birth*

Rats that were big at birth grew up to become big adults (Figure 11 and Table 3). Animals that were small at birth showed no tendency to catch up; rather, the differences between the groups tended to increase over the age span studied. Average body weights of male and female

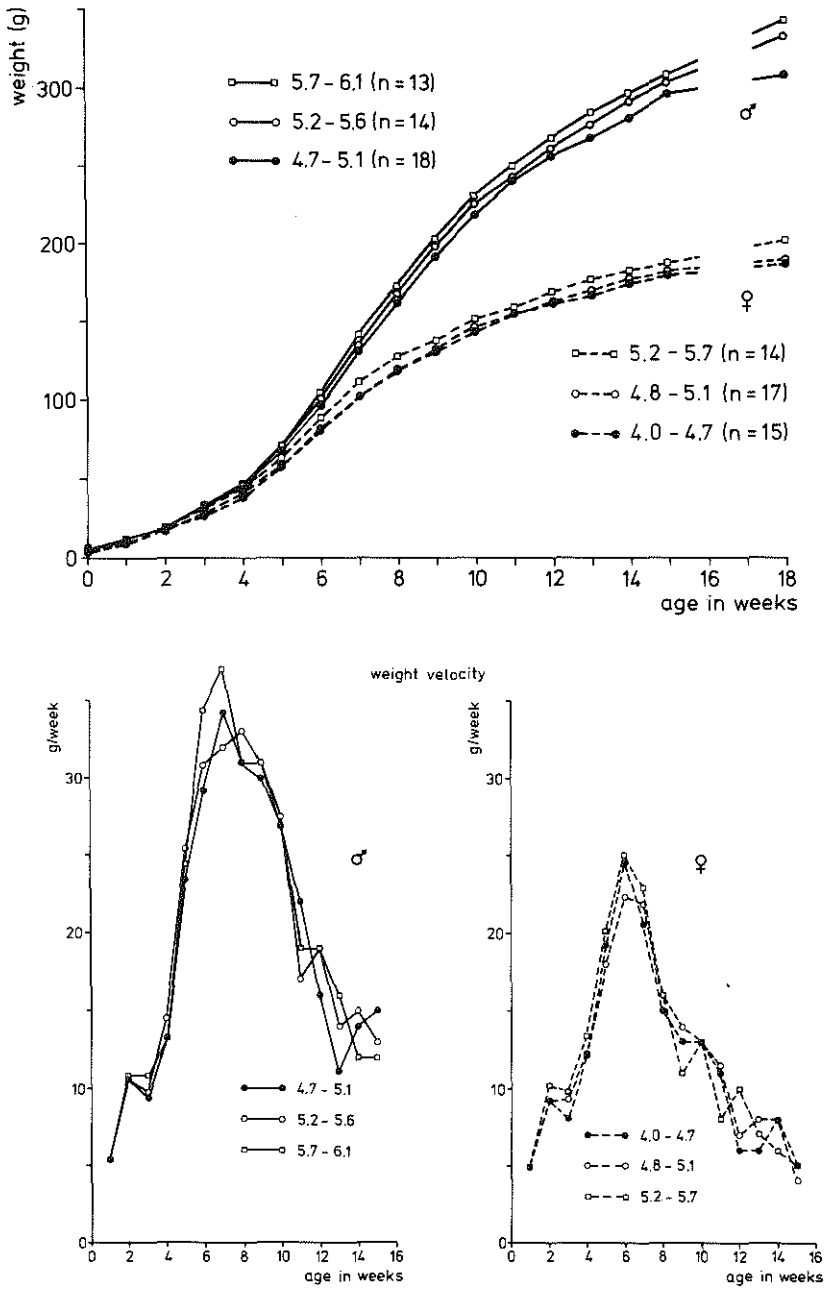



*Figure 11. Untreated male and female rats. Growth in body weight of the animals grouped according to the weight at birth.*

| Sex | Birth weight groups | N | Mean body weight (g) and range | | | |
|---|---|---|---|---|---|---|
| | | | at birth | | at 126 days (18 weeks) | |
| ♂ | low | 18 | 5.0 | (4.7 - 5.1) | 319 | (285 - 377) |
| | high | 13 | 5.8 | (5.7 - 6.1) | 342$^{+}$ | (306 - 397) |
| ♀ | low | 15 | 4.5 | (4.0 - 4.7) | 188 | (174 - 203) |
| | high | 14 | 5.4 | (5.2 - 5.7) | 203$^{++}$ | (180 - 223) |

$^{+}$ significantly different from low birth weight group ($t=2.68$, $p < .02$)

$^{++}$ significantly different from low birth weight group ($t=2.31$, $p < .05$)

*Table 3. Untreated male and female rats. Relationship between body weights at birth and in adulthood.*

rats in adulthood for the heaviest-at-birth and the lightest-at-birth were significantly different (see Table 3). The differences in growth rate were greatest during the first 6 weeks in females and during the first 7 weeks in males, i.e. prepuberally.

Puberty in female rats, as judged from opening of the vagina, was reached between 37 and 48 days of age, with an average age of 43.6 days (Figure 12).

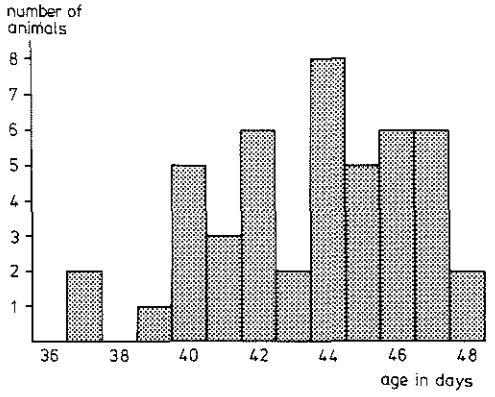



*Figure 12. Untreated female rats. Age at vaginal opening in 46 rats (mean age: 43.6 days).*

No information was available for the onset of puberty in males in the present study. There was no significant correlation between body weight at birth and age at vaginal opening (Table 4). On the basis of the body weight velocity curves a weight difference of about 20 to 25 g was

| Opening vagina (age in days) | | | Body weight at opening vagina | Body weight at birth |
|---|---|---|---|---|
| Range | Mean | N | (g ± S.E.M.) | (g ± S.E.M.) |
| 37 - 41 | 39.6 | 11 | 84.7 ± .82 | 5.04 ± .08 |
| 46 - 48 | 46.7 | 14 | 94.4[a] ± 1.68 | 5.14 ± .15 |

[a] significantly different (U=12, $p < .002$)

*Table 4. Untreated female rats. Relationships between age and body weight at vaginal opening, and body weight at birth. The ranges are the tail ends of the distribution shown in figure 12.*

to be expected between the 2 groups of early and late maturing females in table 3. Actually, the difference in mean body weight was 9.7 g. This clearly demonstrates the well-known phenomenon that in female rats puberty is more closely related to body weight than to age (e.g. Kennedy and Mitra, 1963).

## EXPERIMENT 2. POSTNATALLY GONADECTOMIZED MALE AND FEMALE RATS

### METHODS

#### Procedure and subjects

Pups from 12 litters were used to constitute 10 litters of 8 young on the day of birth, within 12 hours after delivery. Most of the young were left with their own mother. On the day of birth (Day 1) the pups were sexed, weighed, marked with coloured ink and within each litter randomly assigned to one of the 3 groups: (a) gonadectomy on Day 1 and sham operation on Day 21; (b) sham operation on Day 1 and gonadectomy on Day 21; and (c) sham operation on both Days 1 and 21 (designated as "intact" in the following paragraphs).

The young that died during the suckling period were replaced by untreated animals of the same age and left in the litter till the time of weaning.

#### Measurement of physical development

The rats were weighed between 09.00 and 11.00 hours every 7th day until 210 days of age. Body length and tail length were measured on Day 7 and thereafter whenever the animals were weighed till 112 days of age. Re-marking occurred every other day until about 20 days of age when all animals were eartagged. On Days 28, 56, and 91 whole body radiographs were taken under ehter anaesthesia of the animals. Starting on Day 30 the intact females were checked daily for opening of the vagina.

#### Androgen treatment

At 112 days of age animals of each group were assigned, while matched for body weight, to one of 2 groups to be given either testosterone propionate (TP) or oil injections. Starting on Day 115 the TP-groups receiv-

ed a subcutaneous injection of .5 mg TP in .1 ml oil every three days, whereas the oil-group received .1 ml of oil. These injections were continued till Day 182, whereafter the androgen dose was increased to 2.5 mg TP in .1 ml oil every three days up to Day 210. The TP animals thus received a total of 34 mg testosterone propionate over a period of 95 days.

### Behavioural measures

In adulthood the animals were tested for male copulatory behaviour. Specifications of the testing procedure will be presented with the results.

## RESULTS

### Physical measures

*Somatic growth*

The data are shown in figures 13, 14 and 15. Removal of the gonads caused slower growth in males and faster growth in females. Castration at birth or on Day 21 resulted in males that were significantly lighter, with shorter bodies and tails than intact males. There was also a significant difference in each of these measures between Day 1 and Day 21 castrated males; the Day 1 castrates being lighter and smaller in size (see Table 5).

Ovariectomy at birth or at 21 days of age resulted in females that were significantly heavier, with longer bodies and tails than intact females. There were no significant differences in body weight and body length between Day 1 and Day 21 ovariectomized females, although ovariectomy at birth resulted in shorter tails.

The three male body weight curves diverged from Day 35 on, whereas in the females this phenomenon started 2 weeks later (between the sham-operated and the two ovariectomized groups). Intact males and females showed a peak in weight velocity in the 7th and 6th week respectively. This sex difference was reversed in the gonadectomized animals. After

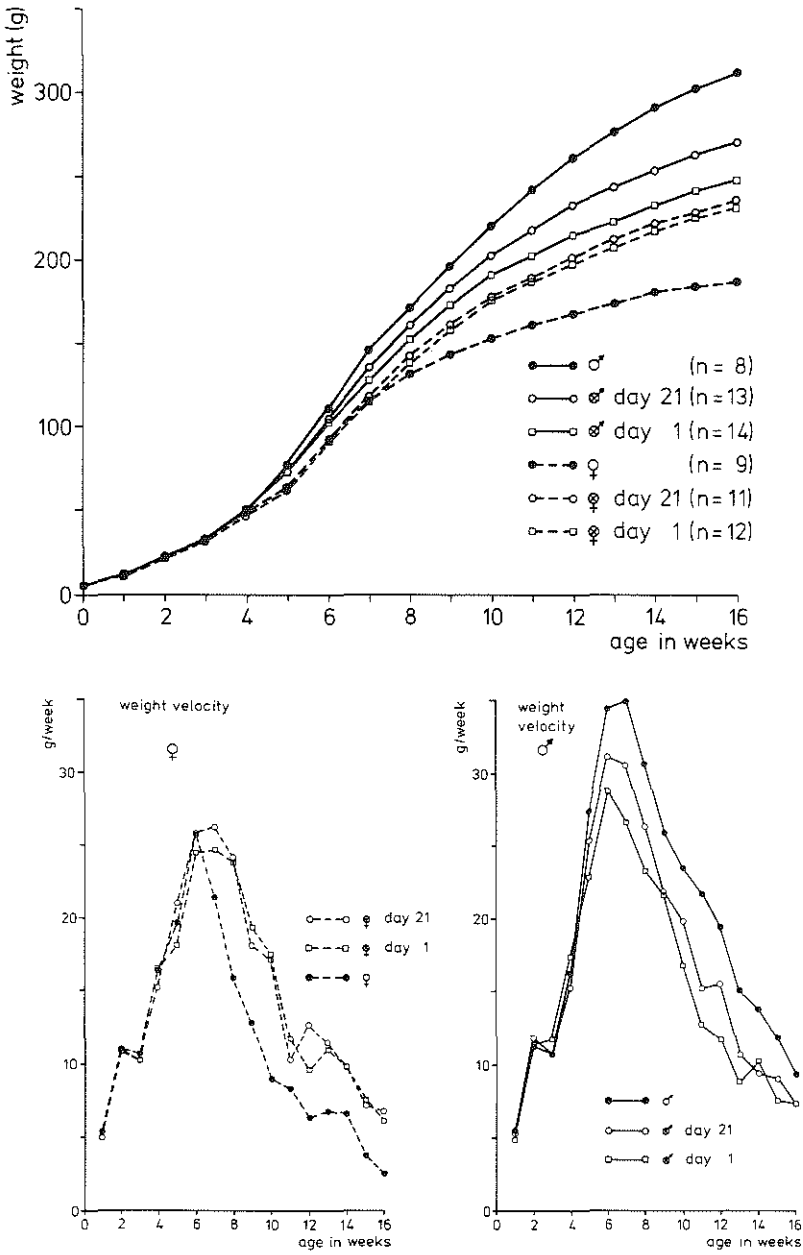



*Figure 13. Postnatally gonadectomized male and female rats. Effects of gonadectomy on Days 1 or 21 on subsequent growth in body weight.*

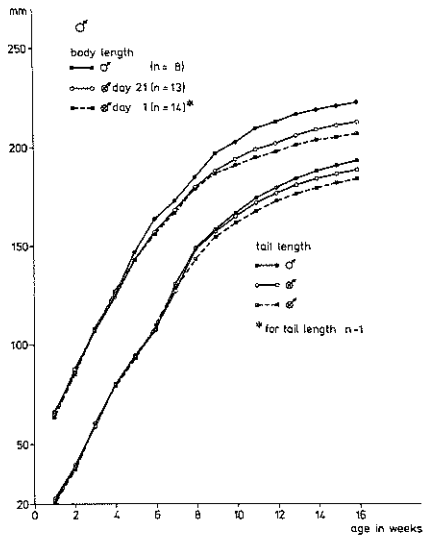



*Figure 14. Postnatally gonadectomized male rats. Effects of castration on Days 1 or 21 on subsequent growth in body length and tail length.*

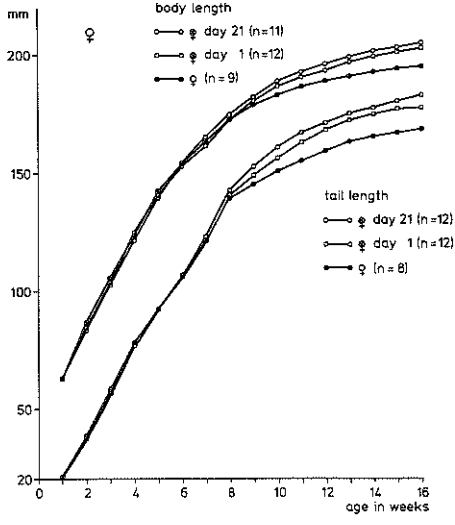



*Figure 15. Postnatally gonadectomized female rats. Effects of ovariectomy on Days 1 or 21 on subsequent growth in body length and tail length.*

| Group comparisons | Body weight | Body length | Tail length |
|---|---|---|---|
| ♂ Sham-castration vs ♂ Castration day 1 | U=2, $p < .002$ | U=1, $p < .002$ | U=12, $p < .02$ |
| ♂ Sham-castration vs ♂ Castration day 21 | U=8, $p < .002$ | U=2.5, $p < .002$ | U=20, p=.02 |
| ♂ Castration day 1 vs ♂ Castration day 21 | U=40, $p < .02$ | U=44.5, $p < .05$ | U=42, $p < .05$ |
| ♀ Sham-ovariectomy vs ♀ Ovariectomy day 1 | U=0, $p < .002$ | U=12, p=.002 | U=1, $p < .002$ |
| ♀ Sham-ovariectomy vs ♀ Ovariectomy day 21 | U=0, $p < .002$ | U=11.5, $p < .02$ | U=0, $p < .002$ |
| ♀ Ovariectomy day 1 vs ♀ Ovariectomy day 21 | U=57.5, ns | U=56, ns | U=27.5, $p < .02$ |
| ♂ Castration day 1 vs ♀ Ovariectomy day 1 | U=77.5, ns | U=51, ns | U=37, $p < .05$ |
| ♂ Castration day 21 vs ♀ Ovariectomy day 21 | U=19, $p < .02$ | U=32, $p < .05$ | U=23.5, $p < .02$ |

*Table 5. Postnatally gonadectomized male and female rats. Statistical analyses of data recorded at 112 days of age (16 weeks).*

the peak in body weight velocity, Day 21 castrated males continued to grow at a higher rate than Day 1 castrates, whereas in the females there was no difference in growth rate between the ovariectomized animals.

The mean age of opening of the vagina (puberty) of sham-operated animals was 39.5 days (range 39 - 41 days).

These data show that gonadectomy at birth caused the disappearance of sex differences in postnatal growth between male and female rats.

| Treatment | N | Sex | Skeletal maturity score | | | N | Length of radius (mm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 28 days | 56 days | 91 days | | 28 days | 56 days | 91 days |
| Castration day 1 | 9 | ♂ | 50.0 | 58.8 | 64.2 | 10 | 14.7 | 19.7 | 22.3 |
| Castration day 21 | 10 | ♂ | 50.0 | 57.6 | 64.2 | 11 | 14.6 | 19.6 | 22.8 |
| Sham-castration | 8 | ♂ | 50.5 | 59.2 | 64.0 | 8 | 14.8 | 19.9 | 23.1$^{+}$ |
| Ovariectomy day 1 | 10 | ♀ | 48.2 | 57.6 | 64.6 | 12 | 14.6 | 19.3 | 22.3 |
| Ovariectomy day 21 | 9 | ♀ | 47.6$^{+++}$ | 57.7 | 64.3 | 12 | 14.4 | 19.1 | 22.3 |
| Sham-ovariectomy | 7 | ♀ | 49.3 | 57.7 | 63.6 | 9 | 14.5 | 19.3 | 21.5$^{++}$ |

$^{+}$ significantly different from day 1 castrated males (U=14.5, $p < .05$) and sham-ovariectomized females (U=1, $p < .002$)

$^{++}$ significantly different from day 1 and day 21 ovariectomized females (U=9 and 12 resp., $p < .002$)

$^{+++}$ significantly different from all male groups ($U \leq 14$, $p < .02$)

*Table 6. Postnatally gonadectomized male and female rats. Gonadectomy on Days 1 or 21. Skeletal maturity and length of radius at various ages.*

*Skeletal maturity*

There were no significant effects of gonadectomy at birth or at 21 days of age on bone maturation in either sex (Table 6). Except for the Day 21 ovariectomized females at 28 days of age there were no differences in skeletal maturity score. Effects of gonadectomy on bone length were apparent at the age of 91 days. At that age intact males had significantly longer bones, as judged from the length of the radius, than either Day 1 castrated males or intact females. The intact females had shorter bones than either Day 1 or Day 21 ovariectomized females.

*Androgen treatment and body weight*

The effects of prolonged TP administration in adulthood on body weight are shown in table 7. The low dose androgen treatment (.5 mg TP/3 days from Day 115 - 182) caused an increase in body weight in Day 21 castrated males ($U = 2$, $p = .004$), and intact females ($U = 0$, $p = .016$). The total androgen treatment (low dose plus high dose from Day 115 - 210) stimulated body weight growth in the intact females ($U = 0$, $p = .016$) only. The low dose, as well as the high dose TP treatment, had a significant body weight growth retarding effect in Day 21 ovariectomized females ($U's = 0$, $p = .016$). Only the total androgen treatment caused a borderline significant growth retardation in females ovariectomized at birth ($U = 5$, $p = .041$ one-tailed). No significant effects of low dose androgen treatment on body weight growth were observed in Day 1 gonadectomized animals and intact males. The total TP treatment did not affect body weight in either Day 21 castrated or intact males.

Following termination of the TP injections, Day 1 and Day 21 ovariectomized females showed a significantly higher increase in body weight than the intact females.

Note that during this treatment deaths only occurred among the Day 1 castrated males treated with TP. Of the 7 animals initially assigned to the TP-group, only 3 survived till 350 days of age; it is not with any certainty attributable to untoward environmental factors operating at that time.

*Androgen treatment and sex behaviour*

In order to establish the effects of TP administration on mating

| Sex | Treatment | | N | Mean body weight (g) at 112 days | Mean increment in body weight (g) | | |
|---|---|---|---|---|---|---|---|
| | Neonatally | In adulthood 115-210 days | | | 112 - 182 days | 112 - 210 days | 210 - 350 days |
| Males | Castration day 1 | TP | 4 | 238 | 47 | a) | a) |
| | Castration day 1 | oil | 7 | 247 | 45.4 | 57.4 | 23.7 |
| | Castration day 21 | TP | 7 | 271 | 76.3+ | 74.7 | 36.6 |
| | Castration day 21 | oil | 6 | 267 | 52.3 | 65 | 26.7 |
| | Sham-castration | TP | 4 | 312 | 57.8 | 58.5 | 64.5 |
| | Sham-castration | oil | 4 | 310 | 66.5 | 79.8 | 40.8 |
| Females | Ovariectomy day 1 | TP | 6 | 230 | 42.5 | 45.2+ | 35.8+ |
| | Ovariectomy day 1 | oil | 5 | 236 | 48 | 62.4 | 11.6 |
| | Ovariectomy day 21 | TP | 5 | 239 | 29+ | 31+ | 34.8+ |
| | Ovariectomy day 21 | oil | 4 | 239 | 44.8 | 55.2 | 19.8 |
| | Sham-ovariectomy | TP | 5 | 188 | 56.8+ | 60.4+ | -6.4 |
| | Sham-ovariectomy | oil | 4 | 186 | 25.4 | 31.4 | 2.0 |

a) no data: 2 animals became sick and lost weight

+ significantly different from oil injected control group ($p < .02$)

*Table 7. Postnatally gonadectomized male and female rats. Gonadectomy on Days 1 or 21. Body weight affected by prolonged administration of TP in adulthood.*

behaviour, all animals were individually tested with 2 receptive females simultaneously. Testing occurred in a semicircular cage with a glass front and a grid floor in a semi-dark observation room. The "stimulus" females had previously been ovariectomized and brought into heat by injections of 6 γ oestradiol benzoate, followed 28 hours later by .5 mg progesterone. The primed females were placed with the experimental animals some 4 hours after the progesterone injection, during the first quarter of the dark cycle. One experimenter observed the androgen or oil treated animals and the male copulatory behaviours were scored. The test was terminated following ejaculation or after 15 minutes if no intromission had occurred by that time. The males were tested twice, once near the end of the low dose TP treatment (176 days of age), and a second time at the end of the high dose TP treatment (210 days of age). Since there were no differences apparent between the scores of the two tests they have been combined in the presentation of the data. The females were tested once at the end of the total androgen treatment (210 days of age).

As can be seen from table 8 the androgen treated gonadectomized animals of either sex displayed more male copulatory behaviours than their oil injected controls. Comparison of male sex behaviours displayed by oil injected gonadectomized male and female rats revealed rather striking differences. Three out of seven Day 1 castrated males and 2 out of 5 Day 1 ovariectomized females showed mounting behaviours, whereas none of the Day 21 castrated males and only 1 out of 4 Day 21 ovariectomized females did so. Of the gonadectomized animals only TP treated Day 21 castrates were capable of showing the complete copulatory pattern (6 out of 7). None of the ovariectomized females of either treatment showed intromission and ejaculation behaviours. There were no significant effects of TP on the behaviours of the intact males and females.

*Autopsy data*

Autopsy was performed at 350 days of age. The results are shown in tables 9 and 10 for the males, and in tables 11 and 12 for the females. Only in intact females does the TP treatment in adulthood seem to have had a lasting effect on body weight: the androgen treated females were heavier than the oil injected controls ($U = 2$, $p = .064$). The effects of gonadectomy on body weight noted at 112 days of age still existed at

| Sex | Treatment | | N | Mounts | Mounts with thrusts | Intromissions | Ejaculation |
|---|---|---|---|---|---|---|---|
| | Neonatally | In adulthood 115–210 days | | | | | |
| Males | Castration day 1 | TP | 4 | 100 (2.0)[+] | 100 (18.1) | 0 | 0 |
| | Castration day 1 | oil | 7 | 43 (1.2) | 14 (4) | 0 | 0 |
| | Castration day 21 | TP | 7 | 86 (3.8) | 100 (31.3) | 86 (6.8) | 86 (1.0) |
| | Castration day 21 | oil | 6 | 0 | 0 | 0 | 0 |
| | Sham-castration | TP | 4 | 75 (2.0) | 75 (21.0) | 75 (4.8) | 75 (1.0) |
| | Sham castration | oil | 4 | 100 (6.6) | 100 (28.0) | 100 (10.8) | 100 (1.0) |
| Females | Ovariectomy day 1 | TP | 6 | 50 (3.0) | 83 (12.2) | 0 | 0 |
| | Ovariectomy day 1 | oil | 5 | 40 (1.5) | 20 (5) | 0 | 0 |
| | Ovariectomy day 21 | TP | 5 | 100 (1.8) | 100 (14.0) | 0 | 0 |
| | Ovariectomy day 21 | oil | 4 | 0 | 25 (13) | 0 | 0 |
| | Sham-ovariectomy | TP | 5 | 60 (3.0) | 40 (22.0) | 0 | 0 |
| | Sham-ovariectomy | oil | 4 | 50 (2.0) | 50 (5.5) | 0 | 0 |

[+] in parentheses the mean score of the animals showing the behaviour

*Table 8. Postnatally gonadectomized male and female rats. Gonadectomy on Days 1 or 21. Percentage of animals showing male copulatory behaviour after prolonged administration of TP in adulthood. Males were tested at 176 and 210 days of age; females were tested at 210 days of age only (one male, castrated on Day 1, was tested only at 176 days of age).*

350 days of age. There were no differences in body weight between animals ovariectomized at birth or at 21 days of age, but both ovariectomized groups were significantly heavier than intact females ($U = 0$, $p < .002$). Males castrated at birth were significantly lighter than either Day 21 castrated or intact males ($U = 9$ and 0 respectively, $p < .002$). Males castrated at 21 days of age were also significantly lighter than intact males ($U = 4.5$, $p < .002$). The weights of specific gonadal hormone "dependent" organs, such as the ventral prostate and the seminal vesicles in males, and the uterus in females, were significantly increased after TP treatment in gonadectomized animals. Although the untreated Day 21 castrated males had also heavier ventral prostates and seminal vesicles than Day 1 castrates, these differences never reached the $p = .05$ level of significance.

| Treatment | | N | Body weight (g) | Liver (g) | Adrenals (mg) | Pituitary (mg) | Ventral prostate (mg) | Seminal vesicles (mg) | Testes (g) |
|---|---|---|---|---|---|---|---|---|---|
| Neonatally | In adulthood 115-210 days | | | | | | | | |
| Castration day 1 | TP | 3 | 297 | 8.60 | 32.3 | 11.1 | 41.3 | 52.0 | |
| Castration day 1 | oil | 7 | 328 | 9.92 | 40.4 | 10.9 | 6.0 | 8.6 | |
| | | | U=9 ns | U=6 ns | U=1 p=.034 | U=10 ns | U=0 p=.016 | U=0 p=.016 | |
| Castration day 21 | TP | 7 | 383 | 12.93 | 36.5 | 12.5 | 52.1 | 63.4 | |
| Castration day 21 | oil | 6 | 360 | 10.86 | 37.0 | 13.7 | 16.4 | 17.3 | |
| | | | U=11 ns | U=9.5 ns | U=17 ns | U=13.5 ns | U=4 p=.014 | U=1 p=.002 | |
| Sham-castration | TP | 4 | 435 | 16.30 | 43.1 | 12.6 | 413.5 | 497.5 | 3.00 |
| Sham-castration | oil | 4 | 431 | 17.42 | 37.2 | 9.3 | 431.2 | 376.4 | 2.95 |
| | | | U=9 ns | U=8 ns | U=7 ns | U=3 ns | U=9 ns | U=3 ns | U=8 ns |

*Table 9. Postnatally gonadectomized male rats. Castration on Days 1 or 21. TP administration in adulthood. Weights at 350 days of age.*

| Treatment | | N | Liver (g) | Adrenals (mg) | Pituitary (mg) | Ventral prostate (mg) | Seminal vesicles (mg) | Testes (g) |
|---|---|---|---|---|---|---|---|---|
| Neonatally | In adulthood 115-210 days | | | | | | | |
| Castration day 1 | TP | 3 | 2.89 | 10.9 | 3.7 | 14.1 | 17.8 | |
| Castration day 1 | oil | 7 | 3.01 | 12.3 | 3.3 | 1.9 | 2.7 | |
| | | | U=6 ns | U=6 ns | U=8 ns | U=0 p=.016 | U=0 p=.016 | |
| Castration day 21 | TP | 7 | 3.37 | 9.6 | 3.3 | 13.8 | 16.6 | |
| Castration day 21 | oil | 6 | 3.02 | 10.3 | 3.8 | 4.2 | 4.9 | |
| | | | U=11 ns | U=14 ns | U=10 ns | U=4 p=.014 | U=1 p=.002 | |
| Sham-castration | TP | 4 | 3.77 | 9.9 | 2.9 | 92.9 | 114.6 | .69 |
| Sham-castration | oil | 4 | 4.02 | 8.6 | 2.1 | 99.5 | 88.6 | .69 |
| | | | U=4 ns | U=2 ns | U=2.5 ns | U=6 ns | U=4 ns | U=7 ns |

*Table 10. Postnatally gonadectomized male rats. Castration on Days 1 or 21. TP administration in adulthood. Organ weights/100 g body weight at 350 days of age.*

| Treatment | | N | Body weight (g) | Liver (g) | Adrenals (mg) | Pituitary (mg) | Uterus (mg) | Ovaries (mg) |
|---|---|---|---|---|---|---|---|---|
| Neonatally | In adulthood 115-210 days | | | | | | | |
| Ovariectomy day 1 | TP | 6 | 311 | 9.09 | 37.9 | 11.8 | 54.6 | |
| Ovariectomy day 1 | oil | 5 | 312 | 9.42 | 35.7 | 12.6 | 27.5 | |
| | | | U=14 ns | U=12 ns | U=9 ns | U=10.5 ns | U=4 p=.052 | |
| Ovariectomy day 21 | TP | 5 | 304 | 8.33 | 33.1 | 11.7 | 50.6 | |
| Ovariectomy day 21 | oil | 4 | 314 | 8.90 | 39.1 | 11.5 | 26.2 | |
| | | | U=8.5 ns | U=8 ns | U=2 p=.064 | U=9 ns | U=0 p=.016 | |
| Sham-ovariectomy | TP | 5 | 242 | 9.16 | 46.0 | 12.3 | 459.3 | 64.8 |
| Sham-ovariectomy | oil | 4 | 219 | 8.54 | 47.0 | 13.5 | 599.1 | 56.6 |
| | | | U=2 p=.064 | U=6 ns | U=9.5 ns | U=5.5 ns | U=5 ns | U=5 ns |

*Table 11. Postnatally gonadectomized female rats. Ovariectomy on Days 1 or 21. TP administration in adulthood. Weights at 350 days of age.*

| Treatment | | N | Liver (g) | Adrenals (mg) | Pituitary (mg) | Uterus (mg) | Ovaries (mg) |
|---|---|---|---|---|---|---|---|
| Neonatally | In adulthood 115-210 days | | | | | | |
| Ovariectomy day 1 | TP | 6 | 2.92 | 12.2 | 3.8 | 17.6 | |
| Ovariectomy day 1 | oil | 5 | 2.99 | 11.4 | 4.0 | 8.8 | |
| | | | U=12 ns | U=9 ns | U=12 ns | U=5 p=.082 | |
| Ovariectomy day 21 | TP | 5 | 2.72 | 10.9 | 3.9 | 16.6 | |
| Ovariectomy day 21 | oil | 4 | 2.84 | 12.4 | 3.7 | 8.3 | |
| | | | U=8 ns | U=2 p=.064 | U=6 ns | U=0 p=.016 | |
| Sham-ovariectomy | TP | 5 | 3.78 | 19.5 | 5.1 | 189.9 | 26.7 |
| Sham-ovariectomy | oil | 4 | 3.92 | 21.5 | 6.2 | 271.1 | 25.9 |
| | | | U=8 ns | U=2 p=.064 | U=3 ns | U=4 ns | U=8 ns |

*Table 12. Postnatally gonadectomized female rats. Ovariectomy on Days 1 or 21. TP administration in adulthood. Organ weights/100 g body weight at 350 days of age.*

# EXPERIMENT 3. FEMALE PSEUDOHERMAPHRODITIC RATS

## METHODS

### Procedure and subjects

The procedure of prenatal androgenization was almost identical to that described by Ward (1969). Fourteen time-mated pregnant rats were injected with 2 mg TP from Day 16 through 20 of gestation. This treatment has previously been reported to permit the birth of viable litters with marked behavioural and considerable somatic masculinization (Gerall and Ward, 1966). About 2 to 3 days following the last TP injection, eleven females gave birth to 101 pups, 20 of which were still-born. Since the external genitalia were masculinized the gonadal sex of the pups had to be ascertained through laparotomy. The female offspring were randomly assigned to one of 2 groups, ovariectomized or sham-operated; these operations were carried out within 12 hours of birth. One half of each group was injected subcutaneously in the neck area with either .05 ml sesame oil or .5 mg TP in .05 ml oil every other day from Day 2 through 20 days of age. The day of birth was designated Day 1. Following the operation the animals were marked with coloured ink, weighed, and placed with a lactating foster mother. The latter because females treated with androgen during pregnancy do not lactate optimally following delivery. The litter size was kept at 9 pups and animals which died during the suckling period were replaced by untreated pups of the same age up till the time of weaning. The mortality caused by the operation and/or postnatal injections was 59%.

### Measurement of physical development

The rats were weighed between 09.00 and 11.00 hours every 7th day until Day 126. Body length and tail length were measured on Day 7 and thereafter whenever the animals were weighed. On Days 28, 56, and 91 whole body radiographs were taken under ether anaesthesia of all the animals.

# RESULTS

## Physical measures

*Somatic growth*

The data are shown in table 13 and figures 16 and 17. The administration of exogenous androgen to prenatal male and female rats resulted in

| | Prenatal TP (days 16-20) | | No treatment | |
|---|---|---|---|---|
| Sex | ♂ | ♀ | ♂ | ♀ |
| Mean body weight at birth (g) | 4.15 | 4.0 | 5.33$^{+}$ | 4.95$^{+}$ |
| Number of animals | 31 | 44 | 48 | 49 |
| Mean littersize (range) | 9.1 (7 - 11) | | 9.8 (7 - 13) | |

$^{+}$ significantly different from all other groups ($p < .001$)

*Table 13. Prenatal TP treatment and body weight at birth.*

a significant decrease in body weight at birth (see Table 13). At birth, prenatally treated male rat pups weighed 78% of the untreated male body weight ($t$ = 10.3, $df$ = 77, $p$ <.001), and prenatally androgenized females weighed 75% of the normal female body weight at birth ($t$ = 8.99, $df$ = 91, $p$ <.001). The significant sex difference in body weight at birth between normal male and female rats ($t$ = 4.48, $df$ = 95, $p$ <.001) was no longer apparent in the prenatally treated animals ($t$ = 1.08, $df$ = 73, n.s.). There were no differences in average litter size.

The administration of prenatal TP to genetic females resulted in a somewhat decreased postnatal growth rate compared with normal females (at 112 days of age: body weight: U = 6, $p$ = .05 one-tailed; body length: U = 9, n.s.; tail length: U = 1, $p$ = .002).

Both ovariectomy at birth and postnatal TP administration resulted in significant increases in body weight and body size in female pseudo-

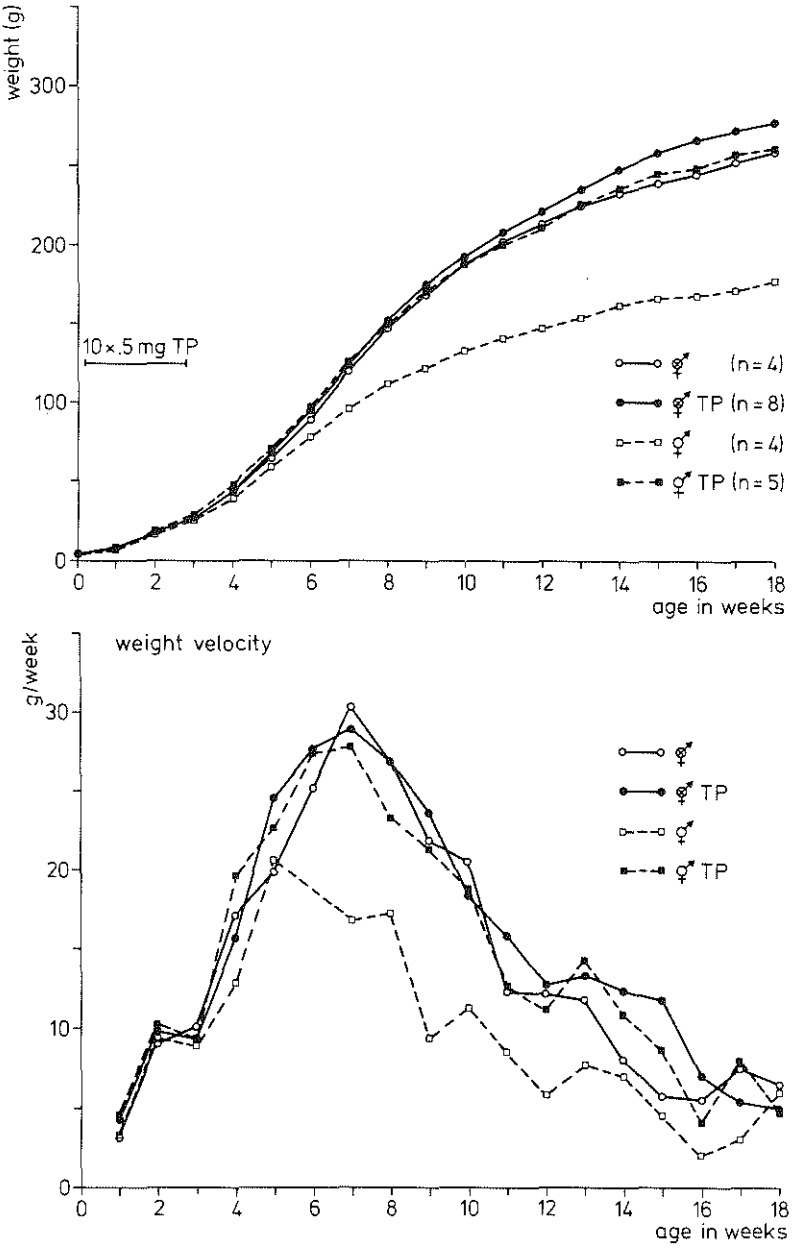



*Figure 16. Female pseudohermaphroditic rats. Effects of ovariectomy on Day 1 and neonatal TP administration on subsequent growth in body weight.*

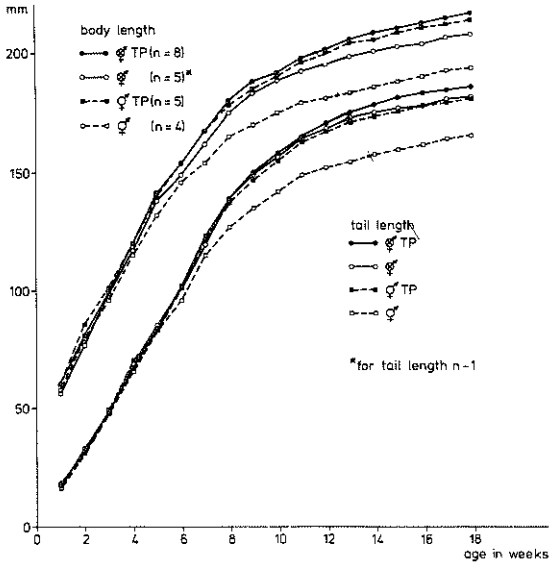



*Figure 17. Female pseudohermaphroditic rats. Effects of ovariectomy on Day 1 and neonatal TP administration on subsequent growth in body length and tail length.*

hermaphrodites (see Table 14). Postnatal administration of TP to Day 1 ovariectomized pseudohermaphrodites did not further significantly stimulate the growth in weight, although there was a (borderline) significant further increase in body length and tail length.

| Group comparisons | Body weight | Body length | Tail length |
|---|---|---|---|
| ♂ Sham-ovariectomy TP vs ♂ Sham-ovariectomy oil | U=0, p=.016 | U=0, p=.016 | U=0, p=.016 |
| ♂ Sham-ovariectomy TP vs ♂ Ovariectomy TP | U=17, ns | U=17, ns | U=12, ns |
| ♂ Ovariectomy TP vs ♂ Ovariectomy oil | U=11, ns | U=6.5, p=.056 | U=6.5, p=.056 |
| ♂ Ovariectomy oil vs ♂ Sham-ovariectomy oil | U=0, p=.028 | U=1.5, p=.048 | U=1, p=.032 |

*Table 14. Female pseudohermaphroditic rats. Statistical analyses of data recorded at 126 days of age (18 weeks).*

*Skeletal maturity*

There were no significant effects of either ovariectomy at birth or postnatal TP administration on bone maturation (Table 15). As judged

| Treatment | | N | Skeletal maturity score | | | Length of radius (mm) | | |
|---|---|---|---|---|---|---|---|---|
| at birth | on days 1-20 | | 28 days | 56 days | 91 days | 28 days | 56 days | 91 days |
| Ovariectomy | TP | 7 | 49.9 | 58.1 | 63.9 | 14.3 | 19.9 | 22.9 |
| Ovariectomy | oil | 3 | 49.3 | 56.7 | 64.0 | 14.6 | 20.1 | 22.6 |
| Sham-ovariectomy | TP | 5 | 48.2 | 58.6 | 63.2 | 14.5 | 19.9 | 22.1 |
| Sham-ovariectomy | oil | 3 | 48.0 | 59.3 | 63.3 | 13.6$^{+}$ | 18.9$^{+}$ | 20.7$^{+}$ |

$^{+}$ significantly different from all other treatment groups (U=0 or 1, $p \leq .036$)

*Table 15. Female pseudohermaphroditic rats. Ovariectomy at birth and prolonged postnatal TP administration. Skeletal maturity and length of radius at various ages.*

from the length of the radius, untreated female pseudohermaphrodites had significantly shorter bones than animals of any other group.

*Autopsy data*

Autopsy was performed at 150 days of age. The results are shown in tables 16 and 17. Examination of the internal structures revealed that the animals possessed heterotypical as well as homotypical organs. All pseudohermaphrodites had a prostate, seminal vesicles (weights of these organs are not presented in the tables), a penis-like clitoris and a levator ani muscle, as well as oviducts, a uterus and ovaries. This corroborates the findings of Ward (1969).

Animals subjected to postnatal androgen treatment were heavier, the difference was only borderline significant in Day 1 ovariectomized animals. Clitoris and levator ani weights were also significantly increased, both absolute as well as relative to body weight. Postnatal TP treatment had caused a significant decrease in ovarian weight, relative to body weight.

Ovariectomy in TP-treated pseudohermaphrodites caused a significant decrease in absolute weights of liver, adrenals and pituitary; this effect was even more striking for the relative weights of these organs. Ovariectomy in oil treated pseudohermaphrodites caused a significant increase in body weight and a significant decrease in weight of the pituitary. Relative to body weight, ovariectomy had a significant decreasing effect on liver, adrenal, and pituitary weight.

| Treatment at birth | Treatment on days 1 - 20 | N | Body weight (g) | Liver (g) | Adrenals (mg) | Pituitary (mg) | Uterus (mg) | Ovaries (mg) | Clitoris (mg) | Levator ani (mg) |
|---|---|---|---|---|---|---|---|---|---|---|
| Ovariectomy | TP | 8 | 307 | 8.54 | 49.6 | 11.4 | 24.5 | | 67.9 | 88.2 |
| Ovariectomy | oil | 5 | 260 | 7.85 | 45.6 | 10.7 | 18.2+ | | 38.4+ | 39.1+ |
| | | | U=7 p=.066 | U=12 ns | U=14 ns | U=11.5 ns | U=7.5 ns | | U=4 p=.048 | U=2 p=.016 |
| Sham-ovariectomy | TP | 5 | 278 | 9.53 | 61.7 | 13.6 | 315.8 | 29.5 | 76.9 | 88.6 |
| Sham-ovariectomy | oil | 3 | 190 | 7.21 | 52.0 | 14.0 | 401.1 | 39.5 | 27.1+ | 22.9+ |
| | | | U=0 p=.036 | U=0 p=.036 | U=3 ns | U=6.5 ns | U=1 p=.072 | U=3 ns | U=0 p=.094 | U=0 p=.094 |
| Statistics: | | | | | | | | | | |
| Ovarex TP vs Sham-ovarex TP | | | U=14 ns | U=7 p=.066 | U=7 p=.066 | U=4 p=.018 | | | U=13 ns | U=20 ns |
| Ovarex oil vs Sham-ovarex oil | | | U=0 p=.036 | U=7 ns | U=5 ns | U=0 p=.036 | | | U=4 ns | U=1.5 ns |

+ number of animals: n-1

*Table 16. Female pseudohermaphroditic rats. Ovariectomy at birth and prolonged postnatal TP administration. Weights at 150 days of age.*

| Treatment at birth | Treatment on days 1 - 20 | N | Liver (g) | Adrenals (mg) | Pituitary (mg) | Uterus (mg) | Ovaries (mg) | Clitoris (mg) | Levator ani (mg) |
|---|---|---|---|---|---|---|---|---|---|
| Ovariectomy | TP | 8 | 2.78 | 16.3 | 3.8 | 8.0 | | 22.3 | 28.6 |
| Ovariectomy | oil | 5 | 2.97 | 17.6 | 4.2 | 7.0+ | | 14.3+ | 15.4+ |
| | | | U=16 ns | U=16 ns | U=6.5 p=.056 | U=11 ns | | U=2 p=.016 | U=3 p=.028 |
| Sham-ovariectomy | TP | 5 | 3.49 | 22.2 | 4.9 | 116.5 | 10.7 | 28.1 | 31.5 |
| Sham-ovariectomy | oil | 3 | 3.80 | 27.6 | 7.5 | 213.8 | 20.7 | 14.3+ | 11.4+ |
| | | | U=3 ns | U=1 p=.072 | U=0 p=.036 | U=0 p=.036 | U=0 p=.036 | U=0 p=.094 | U=0 p=.094 |
| Statistics: | | | | | | | | | |
| Ovarex TP vs Sham ovarex TP | | | U=0 p=.002 | U=0 p=.002 | U=0 p=.002 | U=0 p=.002 | | U=7 p=.066 | U=10 ns |
| Ovarex oil vs Sham-ovarex oil | | | U=1 p=.072 | U=0 p=.036 | U=0 p=.036 | U=0 p=.036 | | U=3 ns | U=3 ns |

+ number of animals: n-1

*Table 17. Female pseudohermaphroditic rats. Ovariectomy at birth and prolonged postnatal TP administration. Organ weights/100 g body weight at 150 days of age.*

# EXPERIMENT 4. NEONATALLY ANDROGENIZED FEMALE RATS

## METHODS

### Procedure and subjects

Within 24 hours after birth, the female offspring of 8 litters were weighed and marked, and randomly assigned to one of two groups, ovariectomized or sham operated. On Day 3 approximately one half of each group was injected subcutaneously in the neck area with either .5 mg TP in .05 ml sesame oil or .05 ml oil. The male offspring was used to keep the litter size at 9 pups. The young that died during the suckling period were replaced by pups of either sex and of the same age. Daily vaginal smears were taken of intact females from Day 130 till autopsy.

### Measurement of physical development

The animals were weighed between 09.00 and 11.00 hours every 7th day until the age of 126 days. Body length and tail length were measured for the first time on Day 7 and thereafter whenever the animals were weighed. On Days 28, 56, and 91 whole body radiographs were taken under ether anaesthesia. Beginning on Day 30 the animals were checked daily for opening of the vagina.

## RESULTS

### Physical measures

*Somatic growth*

The data are shown in figures 18 and 19. At about 6 weeks of age the body weight curves started to diverge; for body length and tail length growth this occurred 1 or 2 weeks later. From the 6th week on the

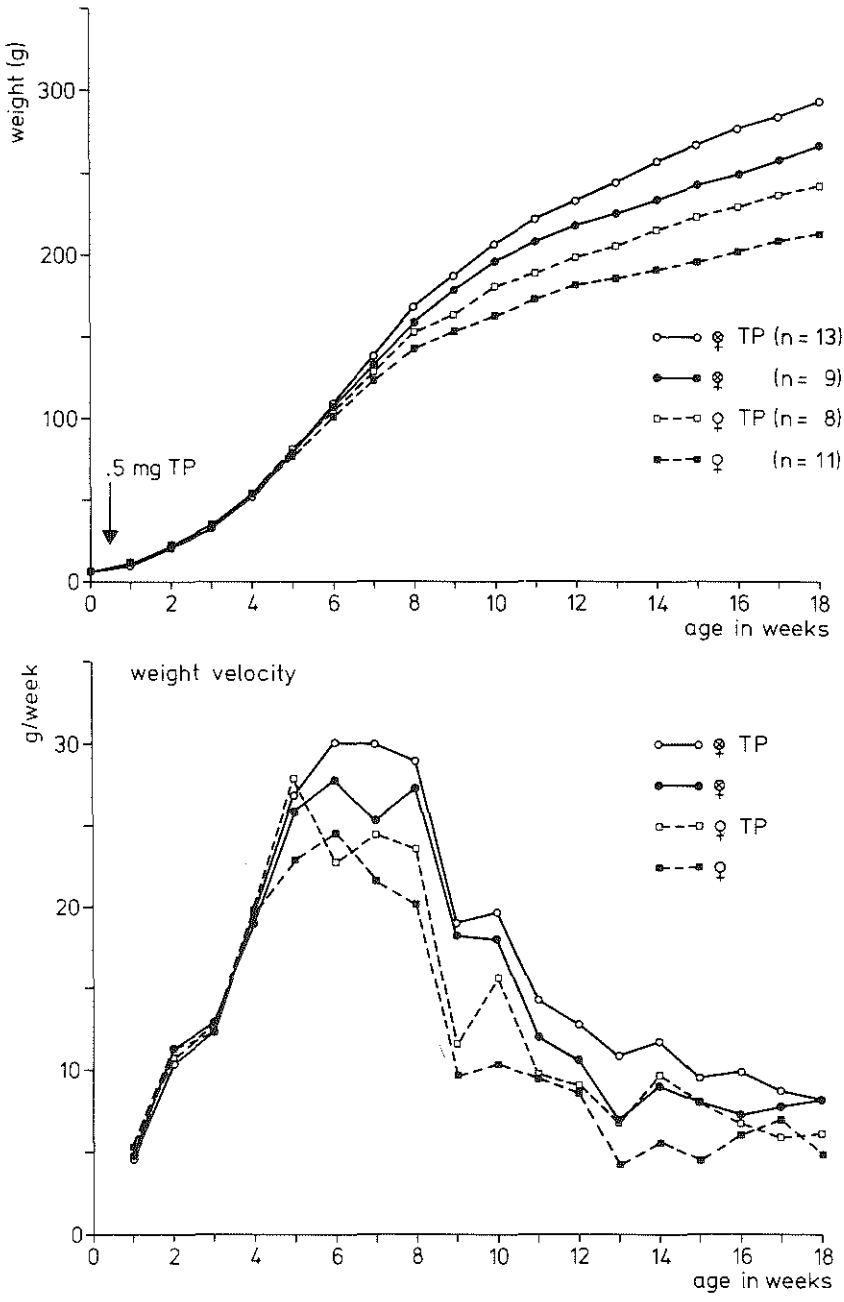



*Figure 18. Neonatally androgenized female rats. Effects of ovariectomy on Day 1 and a single TP injection on Day 3 on subsequent growth in body weight.*

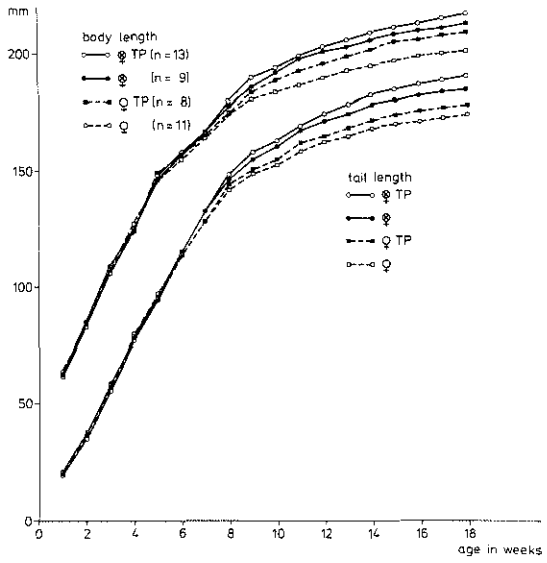



*Figure 19. Neonatally androgenized female rats. Effects of ovariectomy on Day 1 and a single TP injection on Day 3 on subsequent growth in body length and tail length.*

androgenized ovariectomized animals showed a consistently higher growth rate than any other treatment group. Ovariectomy without subsequent androgen injection also caused a significant faster growth after puberty than observed in intact oil injected females (as in Experiment 2). Puberty, as judged from vaginal opening, occurred in the intact oil injected animals at the mean age of 38.5 days (range 35 - 43).

A single TP injection on Day 3 to intact female rats resulted in a significant higher body weight and longer bodies and tails in adulthood than oil injected controls (see Table 18). In Day 1 ovariectomized

| Group comparisons | Body weight | Body length | Tail length |
|---|---|---|---|
| ♀ Sham-ovariectomy TP vs ♀ Sham-ovariectomy oil | U=17.5, $p < .05$ | U=15, p=.02 | U=22, $p < .05$ (one tailed) |
| ♀ Sham-ovariectomy TP vs ♀ Ovariectomy TP | U=9, $p < .002$ | U=20, p=.02 | U=2, $p < .002$ |
| ♀ Ovariectomy TP vs ♀ Ovariectomy oil | U=25, $p < .05$ | U=35, ns | U=28.5, ns |
| ♀ Ovariectomy oil vs ♀ Sham-ovariectomy oil | U=3, $p < .002$ | U=11.5, $p < .02$ | U=7, $p < .002$ |

*Table 18. Neonatally androgenized female rats. Statistical analyses of data recorded at 126 days of age (18 weeks).*

animals the Day 3 TP injection affected the adult body weight, but not the length measures.

*Skeletal maturity*

There was no significant effect of the single TP injection on bone maturation and bone length in either Day 1 ovariectomized or intact females (Table 19). The only significant finding was the fact that Day 1

| Treatment | | N | Skeletal maturity score | | | Length of radius (mm) | | |
|---|---|---|---|---|---|---|---|---|
| at birth | on day 3 | | 28 days | 56 days | 91 days | 28 days | 56 days | 91 days |
| Ovariectomy | TP | 12 | 50.1 | 59.1 | 64.3 | 14.8 | 20.0 | 22.7 |
| Ovariectomy | oil | 7 | 50.3 | 59.0 | 64.4 | 14.9 | 19.8 | 22.4 |
| Combined | | 19 | | | | 14.8 | 19.9 | 22.6 |
| Sham-ovariectomy | TP | 7 | 50.9 | 58.7 | 64.9 | 15.0 | 19.6 | 21.8 |
| Sham-ovariectomy | oil | 8 | 49.9 | 58.1 | 64.0 | 14.7 | 19.3 | 21.4 |
| Combined | | 15 | | | | 14.8 | $19.4^{+}$ | $21.6^{++}$ |

$^{+}$ significantly different from combined ovariectomized animals ($U=83.5$, $p < .05$)

$^{++}$ significantly different from combined ovariectomized animals ($U=32.5$, $p < .002$)

*Table 19. Neonatally androgenized female rats. Ovariectomy at birth and a single TP injection on Day 3. Skeletal maturity and length of radius at various ages.*

ovariectomized females had longer bones than intact animals. This effect, also obtained in Experiment 2, became apparent after puberty (Days 56 and 91).

*Vaginal smears*

The neonatally androgenized females showed persistent vaginal cornification, whereas the oil injected females showed regular cyclic changes of the vaginal epithelium.

*Autopsy data*

Autopsy was performed at the age of 140 days, with the exception of the intact oil injected animals which were autopsied at an average age of 160 days. The results are shown in tables 20 and 21.

The Day 3 TP injection caused a significant decrease in uterine and

ovarian weights, both absolute and relative to body weight. Ovariectomy at birth, regardless of the Day 3 treatment, resulted in an absolute as well as a relative decrease in adrenal and pituitary weight.

| Treatment at birth | Treatment on day 3 | N | Body weight (g) | Liver (g) | Adrenals (mg) | Pituitary (mg) | Uterus (mg) | Ovaries (mg) |
|---|---|---|---|---|---|---|---|---|
| Ovariectomy | TP | 13 | 305 | 9.05++ | 44.2 | 10.9+ | | |
| Ovariectomy | oil | 9 | 274 | 8.86++ | 45.1 | 9.7 | | |
| | | | U=22.5 | U=33 | U=52 | U=31 | | |
| | | | $p < .02$ | ns | ns | ns | | |
| Sham-ovariectomy | TP | 8 | 259 | 9.08 | 69.4 | 14.8 | 354.5 | 36.7 |
| Sham-ovariectomy | oil | 10 | 244 | 7.47 | 61.6 | 12.1 | 490.6 | 74.7 |
| | | | U=10 | U=14 | U=13 | U=21.5 | U=4.5 | U=0 |
| | | | $p < .02$ | $p < .05$ | p=.02 | ns | $p < .002$ | $p < .002$ |
| Statistics: | | | | | | | | |
| Ovarex TP vs Sham-ovarex TP | | | U=11.5 | U=43 | U=3 | U=4 | | |
| | | | $p < .02$ | ns | $p < .002$ | $p < .002$ | | |
| Ovarex oil vs Sham-ovarex oil | | | U=4 | U=12 | U=4 | U=17 | | |
| | | | $p < .002$ | $p < .05$ | $p < .002$ | ns | | |

+ number of animals: n-1

++ number of animals: n-2

*Table 20. Neonatally androgenized female rats. Ovariectomy at birth and a single TP injection on Day 3. Weights at 140 days of age except the sham-ovariectomized oil injected group; the latter were 162 (range 142 - 178) days old.*

| Treatment at birth | Treatment on day 3 | N | Liver (g) | Adrenals (mg) | Pituitary (mg) | Uterus (mg) | Ovaries (mg) |
|---|---|---|---|---|---|---|---|
| Ovariectomy | TP | 13 | 2.91++ | 14.6 | 3.5+ | | |
| Ovariectomy | oil | 9 | 3.16++ | 16.4 | 3.5+ | | |
| | | | U=10.5 | U=32.5 | U=38.5 | | |
| | | | $p < .02$ | $p < .05$ | ns | | |
| Sham-ovariectomy | TP | 8 | 3.49 | 27.0 | 5.7 | 137.5 | 14.2 |
| Sham-ovariectomy | oil | 10 | 3.38 | 28.0 | 5.4 | 222.6 | 33.6 |
| | | | U=30 | U=29 | U=36 | U=1 | U=0 |
| | | | ns | ns | ns | $p < .002$ | $p < .002$ |
| Statistics: | | | | | | | |
| Ovarex TP vs Sham-ovarex TP | | | U=11.5 | U=0 | U=0 | | |
| | | | $p < .02$ | $p < .002$ | $p < .002$ | | |
| Ovarex oil vs Sham-ovarex oil | | | U=34 | U=0 | U=12 | | |
| | | | ns | $p < .002$ | $p < .05$ | | |

+ number of animals: n-1

++ number of animals: n-2

*Table 21. Neonatally androgenized female rats. Ovariectomy at birth and a single TP injection on Day 3. Organ weights/100 g body weight at 140 days of age except the sham-ovariectomized oil injected group; the latter were 162 (range 142 - 178) days old.*

## DISCUSSION OF PART A

The main findings from the above experiments may be listed first.

1. There exists a positive correlation between body weight at birth and body weight in adulthood in male and female rats.
2. There is a sex difference in body growth which sets in from the 5th week onward (around the time of puberty) and which is due to a persistent difference in the rate of growth.
3. Gonadectomy within 24 hours of birth abolishes sex differences in body growth. This effect comes about by a postpuberal fall in the growth rate of males and a rise in the growth rate of females, in comparison with intact animals.
4. Gonadectomy on Day 21 does not abolish sex differences in body growth, although it does cause a diminution of the sex differences.
5. Males castrated at birth become lighter and smaller than males castrated on Day 21; in females there are no differences in growth pattern between Day 1 and Day 21 ovariectomized animals.
6. Prolonged administration of *low* doses of androgen in adulthood causes an increase in body weight growth in Day 21 castrated males and intact females, a decrease in Day 21 ovariectomized females, and no changes in males and females gonadectomized at birth and in intact males. Prolonged treatment with *high* doses of androgen causes cessation of growth in all animals, except intact females. No conclusive data are available in Day 1 castrated males since 5 out of 7 became ill or died during prolonged androgen treatment.
7. Prolonged administration of androgen in adulthood causes a significant increase in the display of male sex behaviour in gonadectomized animals. Males castrated on Day 21 display the complete copulatory pattern, whereas Day 1 castrated males and ovariectomized females do not display intromission and ejaculation behaviour after androgen treatment.
8. Prenatal exposure to androgen causes male and female rats to be born with subnormal body weights.
9. In female pseudohermaphrodites ovariectomy at birth and prolonged neonatal androgen treatment (Days 2 - 20) each cause an increase in the rate of body growth.
10. In female pseudohermaphrodites the combined procedures of ovariectomy

at birth, and prolonged neonatal androgen treatment (Days 2 - 20) result in a growth pattern similar to that of male rats castrated at 21 days of age.

11. A single androgen injection on Day 3 causes intact females to become heavier and larger than oil injected controls. In females ovariectomized at birth such treatment also stimulates growth in weight, but not significantly the growth in length.
12. In females the combined procedures of ovariectomy at birth and a single androgen injection on Day 3 result in a growth pattern similar to that of male rats castrated at 21 days of age.

In the present study it has been found that in male and female rats *body weight at birth* was positively correlated with *body weight in adulthood* (126 days of age) (*Experiment 1*). In other words rats that were big at birth became big adults, while animals small at birth became small adults without showing any kind of catch up growth. This corroborates the findings in the guinea pig (Lister and McCance, 1965; Slob, Goy and Van der Werff ten Bosch, 1972). In the latter investigation it appeared necessary to match groups for body weight at birth in longitudinal studies of postnatal growth and development. While the differences in birth weights are quite striking in the guinea pig, these differences are less conspicuous in the rat. Nevertheless, it should be emphasized that also in the rat, particularly in studies into the effects of prenatal treatments on postnatal growth and development, the experimental and control groups should be matched for body weight at birth. This would make the results of such experiments much more reliable. Studies hitherto presented (see review by Delost, 1971) are not very convincing since their outcome has been biased by the (sometimes significant) differences in body weight at birth.

It has also been shown in the present study that male and female rats *gonadectomized at birth* did not significantly differ in body size at 112 days of age, whereas *gonadectomy on Day 21* only partially abolished the sex difference (*Experiment 2*). Other investigators have reported that in the male rat androgens are secreted throughout the entire prepuberal period (Resko, Feder and Goy, 1968). Therefore, androgens secreted by the prepuberal testes could presumably have organized future body growth so that sex differences would continue to appear in animals gonadecto-

mized on Day 21, independently of subsequent hormonal influences. The significant role of the prepuberal testes was also established by the finding that males castrated on Day 21 became significantly heavier and larger than males castrated at birth. The ovaries did not seem to play an organizing role during neonatal life since ovariectomy at birth and on Day 21 affected growth patterns in identical ways.

The disappearance of the sex dimorphism in rat body growth following gonadectomy at birth, corroborates the findings recently presented by Harvey, Hervey and Hutchinson (1972), and those published several years ago by Grunt (1964b). It should be emphasized, however, that in the latter experiment sham-operated controls were pair-fed to the appropriate gonadectomized animals from the time of weaning. As a result of this procedure, presumably, males castrated at birth were found not to differ significantly from sham-operated controls in body size at 180 days of age. Subsequently Grunt reached the following, rather premature conclusion: ".... that sex differences of length and weight in the rat are primarily due to ovarian inhibition rather than to testicular enhancement". However, the adult body weight of the sham-operated control males was about 350 g, whereas the body weight of *ad lib* fed control males (same strain) in another experiment (Grunt, 1964a) was about 445 g at the same age. There is as yet not sufficient information available about the effects of neonatal castration on food intake, motor activity, and thermoregulatory behaviours in adult male rats (Wade, 1972). Therefore, the only conclusion that could be drawn from the above experiment (Grunt, 1964b) is that pair feeding to Day 1 castrated males inhibited the growth of *intact* male rats. Pair feeding does not seem to be the appropriate method for examining the role of androgens in the normal development of sex differences in growth. In the case of female rats this might be different. Pair feeding did not prevent the body weight increase after ovariectomy at birth (Grunt, 1964b). Comparison of the body weights of the sham-operated females (260 g) with the body weights of *ad lib* fed sham-operated females (275 g) in another experiment (Grunt, 1964a) revealed no striking differences. It is well known that ovariectomy causes an increase in food intake and a decrease in motor activity (Wade, 1972). In the pair feeding situation the ovariectomized female could not increase her food intake. It would seem, therefore, that ovariectomy, i.e. the absence of gonadal oestrogens, must have caused better overall utilization of the food and/or a

decrease of the expenditure (activity).

The results of experiment 2 are contradictory to findings of Grunt (1964a), and Bell and Zucker (1971). Grunt showed that following gonadectomy on Day 25 females became heavier and larger, whereas male rats became lighter and smaller than their appropriate sham-operated controls. Furthermore, he showed that gonadectomy on Day 25 abolished the sex differences in subsequent growth. However, the animals were not matched for body weight and total body length at the time the operations were carried out. This may have biased the outcome of the experiment. The initial mean body weight of ovariectomized females was 41 g (Day 25), whereas the castrated males weighed 36 g (Day 30). For total body lengths (nose to tip of tail) these figures were 20.5 and 19 cm respectively. At the end of the experiment (180 days of age) the weight and length of ovariectomized females were 363 g and 43.5 cm. For castrated males the figures were 366 g and 43 cm. Thus males castrated on Day 25 must have had a higher growth rate than females ovariectomized on Day 25. In the present study there were no sex differences in the rate of body growth during the first 4 weeks of life; from the 5th to the 12th week males castrated on Day 25 grew consistently at a higher rate than likewise operated females.

The discrepancy between the results of the present study and the findings of Bell and Zucker (1971) may also have a simple (?) explanation. Bell and Zucker reported that from Day 75 onward males castrated at birth were significantly heavier than females ovariectomized on Day 4. However, at the time they matched these gonadectomized animals for body weight, on Day 26, there already existed a difference between the females (mean body weight: 59 g; range 48 - 66) and the males (mean body weight: 63 g; range 49 - 74). This difference in body weight may have biased the outcome of their experiment. Moreover, it is difficult to understand how these authors could assign animals to the various treatment groups on Day 26 as is stated in their paper, when the same numbers of animals had apparently already been assigned to the various neonatal treatments at the time of birth!

It is concluded from the present study that in the rat only postnatal effects of gonadal hormones determine the development of adult sex differences in body weight and body length. The arguments for a prenatal contribution by the gonads, such as those put forward by Bell and Zucker (1971) are not convincing.

The *low* dose *androgen treatment* in adulthood had a stimulatory effect on *body weight* growth in intact females and in males castrated on Day 21, no effect in animals gonadectomized at birth and in intact males, but an inhibitory effect in females ovariectomized on Day 21. The *high* dose androgen treatment caused a complete cessation of body weight growth in all groups, except in intact females which grew as well as their oil injected controls. Some of the findings confirm data reported on *male rats* by Rubinstein and Solomon (1940, 1941). They showed that in prepuberally castrated males a low dose of androgen had a growth promoting effect, whereas a high dose resulted in growth retardation. The results of the present study support the hypothesis that neonatal androgens are essential for the "organization" of neural mechanisms underlying eating and weight regulation in the male rat (Valenstein, Cox and Kakolewski, 1969; Beatty, Powley and Keesy, 1970; Bell and Zucker, 1971; Wade, 1972): Day 21 castrated males, which had been exposed neonatally to testicular androgens, could "respond positively" to low (physiological ?) doses of androgen in adulthood, whereas animals of either sex gonadectomized at birth could not. In females the situation is different. In intact females the continuous androgen administration presumably depressed the output of pituitary gonadotrophins and, through this effect, the ovarian secretion of oestrogens. It is well known that oestrogens inhibit food intake and stimulate locomotor activity, both of which effects tend to inhibit growth (Wade, 1972). In the present study ovarian inactivity brought about by low dose androgen administration nullified these growth inhibiting effects, resulting in an increase in body weight. The growth retarding effect in females ovariectomized on Day 21 can not be explained.

The high dose androgen treatment blocked body growth almost completely in all animals. This corroborates the findings of Harvey, Hervey and Hutchinson (1972). Over the period following the last androgen or oil injection in adulthood both of the previously TP-treated ovariectomized groups showed a significantly higher growth rate than their controls (a similar trend was noted in various groups of males). This suggests that during the androgen treatment, at least during the high dose administration, these animals had been "functionally food-deprived". Therefore, these animals could display catch up growth, a phenomenon well known to occur in the rat following temporary food deprivation in late infancy and adulthood (e.g. Widdowson and McCance, 1963). A lasting

effect of androgen administration was observed in intact females only; the extra weight gained during low dose androgen administration was added to by a normal growth rate during high dose androgen treatment and a slight weight loss during the post-treatment period.

For mounting behaviours in male and female rats testicular androgens are not required, either in the neonatal period, or in adulthood (see also Beach, 1971). However, when *androgens* were administered *in adulthood* these caused a significant increase in *mounting behaviours* both in Day 1 and Day 21 gonadectomized male and female rats, and in intact females. It is remarkable that in the present study oil injected males castrated on Day 21 did not display any mounting behaviours at all. This unusual finding can not be explained.

Of all gonadectomized animals only males castrated on Day 21 displayed the *intromission* and *ejaculation behaviours* in response to androgen treatment. One possible explanation for the absence of intromission and ejaculation behaviours in the other animals could be that the neural mechanisms underlying these two behaviours have not been "organized" or "sensitized" by neonatal testicular androgens, to respond to the "activating" influence of androgens in adulthood (Phoenix, Goy, Gerall and Young, 1959). Another possible explanation which may be more valid for this particular issue is that the lack of intromission and ejaculation behaviours in the present study was primarily due to the small size of the penis, and the consequent limitation of penile stimulation, in Day 1 castrated males and all females (Beach and Holz, 1946; Beach, Noble and Ordnoff, 1969; Nadler, 1969). It has been shown that when Day 1 castrated males are given testosterone in adulthood they fail to exhibit penile growth comparable to that shown by males castrated at 21 days of age. In the latter group the penis was only slightly less responsive to testosterone than in males castrated on Day 50 or later in life (Beach and Holz, 1946; Beach, 1971). It has also been shown that the injection of testosterone into castrated male rats within 96 hours of birth resulted in increased sensitivity of the cornified papillae on the surface of the penis to testosterone injected in adulthood (Mullins and Levine, 1969).

Another interesting phenomenon has been found in the present study, i.e. intact males treated with TP in adulthood required fewer intromissions prior to ejaculation than oil injected controls ($U = 0$, $p < .002$). Further, TP-treated Day 21 castrated males displayed fewer

intromissions prior to ejaculation than oil injected intact males ($U = 4$, $p < .002$), and were not different from TP-treated intact males ($U = 18$, n.s.). A possible explanation for this phenomenon could be that the exogenous androgen had raised the sensitivity of sensory receptors in the penis. Such an effect of TP has also been found by Baum (personal communication, 1972). He observed that TP administration (1 mg/day) to prepuberal male rats from Day 14 onward accelerated the first occurrence of intromission and ejaculation behaviours by 10 days, compared to oil injected controls. On the day the animals ejaculated for the first time, TP-treated males displayed a median number of 17 intromissions, while oil injected animals displayed a median of 24 intromissions prior to ejaculation ($U = 48.5$, $p < .05$).

The exposure to exogenous androgen during the last days of prenatal life caused *female rats* to be highly *masculinized* at birth (*Experiment 3*). Moreover, this treatment also resulted in a significantly decreased body weight at birth in both male and female rats. There is no easy explanation for the latter finding. It confirms the results of Jost (1960) for the rat, and it has also been described to occur in the mouse (Delost, 1971). In the guinea pig equivocal results have been reported as to body weight at birth and the administration of androgens prenatally. Goy, Phoenix and Meidinger (1967) reported a decreased body weight in males and females (TP from days 15 or 24 through days 66 or 60 of pregnancy), whereas Slob, Goy and Van der Werff ten Bosch (1972) found no changes in female body weight, but an increased body weight at birth of male guinea pigs (TP from days 24 through 41 of pregnancy). A possible explanation may be that the prolonged administration of androgen (as well as other hormones and other substances like antibiotics) close to the time of parturition causes "damage" to the placenta and subsequently causes nutritional deprivation of the foetus. Supporting evidence for this hypothesis may be derived from Ch. Jean (cited in Delost, 1971) who reported a 20 - 40% decrease in placental weight of mice, following prenatal oestrogen treatment. Furthermore, Goy, Phoenix and Meidinger (1967) reported a 26.2% abortion rate in TP-treated guinea pigs as compared with a spontaneous abortion rate of 8.3%. Gerall and Ward (1966) reported the lowest percentage of "successful parturitions" in rats when androgen was administered during days 14 - 22 of pregnancy, compared to androgen administration during days 16 - 22 or 16 - 20.

It has been found in the present study that prenatal androgen caused females to grow at a lower rate postnatally than normal females. This confirms the findings of Ward (1969) who showed that at 100 days of age female pseudohermaphroditic rats had a significantly lower body weight than normal females. This lower postnatal rate of growth was not caused by an alteration of the neural growth regulating mechanisms, but was probably due to the fact that the initial body weight, and presumably body size also, was grossly subnormal (see Experiment 1).

Unexpectedly, ovariectomy at birth in female pseudohermaphrodites resulted in an adult body weight similar to that of normal females ovariectomized at birth. This indicates that in prenatally androgenized females the ovaries still possessed their growth inhibiting activity. In fact, these ovaries may have been more active in this respect than normal ovaries, since their removal allowed catch up growth to take place.

The finding that ovariectomy at birth resulted in postnatal growth not significantly different from that of Day 1 gonadectomized male and female rats, demonstrated that prenatal androgen had not "organized" the neural growth regulating mechanisms. This is further evidence in support of the hypothesis that *only* neonatal androgens are essential for this neural "organization": androgen treatment from Days 2 - 20 in Day 1 ovariectomized female pseudohermaphrodites resulted in a growth pattern similar to that of males castrated on Day 21. Moreover, Ward (1969) has reported that female pseudohermaphrodites treated with TP from Days 1 - 40 and ovariectomized on Day 55 had body weights similar to those of normal males at 100 days of age.

*Neonatal androgenization* of *female rats* with a single high dose TP injection on Day 3 has been found to accelerate body growth (body weight as well as body length and tail length) from the 5th week onward (*Experiment 4*). This confirms the results of many other investigators (Barraclough, 1961; Swanson and Van der Werff ten Bosch, 1963; Harris and Levine, 1965; Drach, Cox, Kakolewski and Valenstein, cited in Valenstein, 1968; Beatty, Powley and Keesey, 1970; Bell and Zucker, 1971). Since ovarian and uterine weights were also reduced, one possible explanation could be that this increased body weight arised from a reduction in ovarian oestrogen secretion (Swanson and Van der Werff ten Bosch, 1963). An alternative explanation may be that the sensitivity of neural growth regulating mechanisms to oestrogens is reduced after neonatal androgen-

ization. Beatty, Powley and Keesey (1970) have reported that female rats treated with TP on Day 3 were less sensitive than control females to the weight suppressing effects of oestradiol benzoate in adulthood.

In animals ovariectomized at birth a single high dose TP injection on Day 3 also resulted in increased body size at 126 days of age, and this effect came about through an accelerated "postpuberal" growth rate. This corroborates recent findings of Bell and Zucker (1971) and Ostrow (1971). It should be noted that female rats ovariectomized at birth and androgen treated on Day 3 showed a growth pattern similar to that of males castrated at days 21 of age.

It has previously been reported that male rats injected with a high dose of TP on Days 2 or 5 showed a decreased body growth as well as smaller testes and accessory organs (Swanson and Van der Werff ten Bosch, 1963). Bell and Zucker (1971) reported that a single TP injection on Day 5 to males castrated at birth increased body weight growth, but not significantly so over the time span the animals were studied (up till 107 days of age). Since in the latter study the likewise treated female rats did not become significantly heavier than oil injected controls until the age of 110 days, the differences in body weight in the males would probably also have become significantly different at a later age.

## CONCLUSIONS OF PART A

The above data suggest that at birth both male and female rat brains are not yet "organized" as to the neural mechanisms regulating the sexual dimorphism in the postnatal growth pattern. After birth androgens produced by the prepuberal testes are responsible for the organization of the male growth pattern in the rat. The prepuberal ovaries have no organizing effect. After puberty testicular secretions stimulate, whereas ovarian secretions inhibit body growth.

*Rats undernourished during the suckling period grow very slowly. Above: a food-deprived and a well-fed littermate control female at Day 25. Opposite: a similar pair at Day 250. Note that a difference in body size persists into adulthood, despite ad libitum feeding from Day 25 onward.*

## B. GROWTH AND DEVELOPMENT OF MALE AND FEMALE RATS FOLLOWING VARIOUS REGIMENS OF NEONATAL FOOD DEPRIVATION

### INTRODUCTION

One difficulty in interpreting the results of studies of early undernutrition or malnutrition in rats arises from the kinds of regimens used for producing food deprivation. These regimens (reviewed by Altman, Das and Sudarshan, 1970; Plaut, 1970) rely on such techniques as (a) removing the infant from the mother for various lengths of time, (b) combining litters so that 15 - 20 pups must suckle from one mother, and (c) underfeeding the mother during gestation and/or lactation. All of these regimens are effective in producing smaller animals. As adults these animals also show behavioural deficits and deviations in tests of emotionality (Cowley and Griesel, 1964; Franková and Barnes, 1968a; Guthrie, 1968; Franková, 1970a, b; Levitsky and Barnes, 1970), motor ability (Altman, Sudarshan, Das, McCormick and Barnes, 1971; Dobbing, 1971b), and learning (Cowley and Griesel, 1959, 1963, 1966; Lát, Widdowson and McCance, 1960; Barnes, Cunnold, Zimmermann, Simons, MacLeod and Krook, 1966; Franková and Barnes, 1968b; Howard and Granoff, 1968; Levitsky and Barnes, 1970). However, the food deprivation effects can not be completely divorced from the effects of differences in maternal care and social environment.

Early social and maternal deprivation produce deficits in adult sex behaviour (Gruendel and Arnold, 1969) and in adult learning (Nováková, 1966; Fuller, 1967). Female rats underfed during lactation show impaired maternal behaviour (Franková, 1971); rats underfed up to the time of parturition may also be poorer mothers, at least for some days until they recover from the food deprivation (Smart, 1971).

Litter size by itself is known to affect both the quality of maternal care offered (Seitz, 1958; Grota and Ader, 1969) and the behaviour of the young once they have become adults (Seitz, 1954).

In order to determine the behavioural effects of food deprivation *per se* it is necessary to study food-deprived animals reared in same-sized litters and with the same quality of maternal care as their well-fed controls. In the present study an attempt was made to cause food

deprivation by putting rat pups for 12 hours per day with a foster mother who would display all maternal behaviours except lactation and by maintaining them in standard sized litters (Experiment 1. Foster mother food deprivation).

In order to evaluate the necessity of permanent maternal care during neonatal food deprivation, a second experiment was carried out in which the rat pups were put in an incubator during their daily food deprivation period. Litter size was again kept constant (Experiment 2. Incubator food deprivation).

Finally an attempt was made to assess the contribution of early social interaction with littermates during neonatal food deprivation by increasing litter size to 18 pups (Experiment 3. Big litter food deprivation).

Figure 20 summarizes the designs of the three experiments. Experiments 2 and 3 were carried out simultaneously, one year after experiment 1.

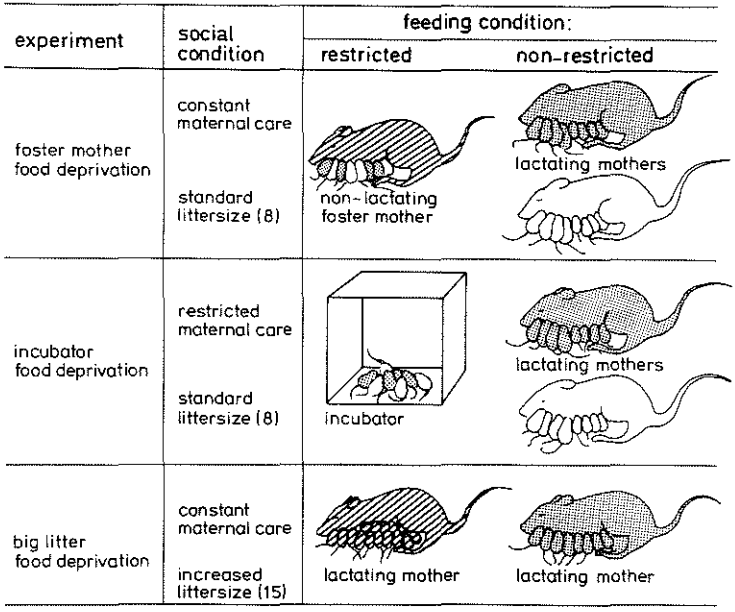



*Figure 20. Outline showing the variation in social condition (maternal care and litter size) in three different food deprivation regimens.*

# EXPERIMENT 1. FOSTER MOTHER FOOD DEPRIVATION

## METHODS

### Deprivation regimen

Thirty-five female rats were placed with males simultaneously so that several litters would be born on one day. Starting about 2 weeks before the expected parturition date, 8 ovariectomized females (future foster mothers) were exposed daily to rat pups, following the priming procedure described by Rosenblatt (1967) for producing maternal behaviour. On the day of birth (Day 1) pups of 33 litters were weighed, sexed, and marked with coloured ink. One day later, young from 2 or, in a few cases, 3 litters were combined to constitute one mixed litter of 12 pups. Such a litter comprised 3 groups: Day animals, Night animals, and Control animals; each group consisted of 2 males and 2 females. Beginning on Day 3, the Nights animals were placed with a foster mother for the light period (09.00 - 21.00 hours) and returned to the lactating mother for the dark period (21.00 - 09.00 hours). The Day animals spent the light period with the lactating mothers and the dark period with the foster mothers. The control animals remained with the lactating mother at all times except for short periods at 09.00 hours and at 21.00 hours, when they were taken out of the nest during the removal of the experimental animals, and for weighing and re-marking. Experimental animals from 2 mixed litters were placed with one foster mother so that each lactating mother and each foster mother was caring for 8 animals at all times.

### Measurement of physical development

The rats were weighed and measured between 09.00 and 10.00 hours. The animals were weighed every other day during the first 10 days of life, every 5th day until Day 25, and thereafter every 10th day until Day 115. Body length and tail length were measured on Day 6 and thereafter whenever the animals were weighed.

Beginning on Day 11 all animals were checked daily for (a) eye

opening; (b) the occurrence of the auditory reflex (startle reflex to a sharp whistle sound); and (c) for the occurrence of the landing reflex (landing on all feet when dropped upside down from a distance of 30 cm to a soft surface). Starting on Day 45 females were checked daily for vaginal opening. On Day 20 and 60 whole body radiographs were taken.

### Behavioural measures

In adulthood subgroups of the animals were subjected to different tests (see General Introduction, Table 1). Care was taken that the tests were administered in the same order for experimental and control animals, since it is known that enriched experience can improve problem-solving ability in the rat (Hebb, 1949, cited in Rosenzweig, 1971). Food-deprived and control animals of either sex were tested at the same time.

## RESULTS

### Physical measures

*Somatic growth*

The data are shown in figures 21, 22 and 23. The food deprivation regimen produced animals which were significantly lighter ($F$ = 131.0, $df$ = 1/74, $p$ <.001), with shorter bodies ($F$ = 189.0, $df$ = 1/74, $p$ <.001) and tails ($F$ = 130.8, $df$ = 1/74, $p$ <.001). Males and females differed significantly on each measure (body weight, $F$ = 172.0, $df$ = 1/74, $p$ <.001; body length, $F$ = 62.1, $df$ = 1/74, $p$ <.001; tail length, $F$ = 58.3, $df$ = 1/74, $p$ <.001). The significant Sex X Nutrition interactions (body weight, $F$ = 24.0, $df$ = 1/74, $p$ <.01; body length, $F$ = 11.0, $df$ = 1/74, $p$ <.01; tail length, $F$ = 7.2, $df$ = 1/74, $p$ <.01) reflected the more serious effect of food deprivation on growth in males than in females. Eye opening, auditory reflex, and landing reflex as well as vaginal opening were significantly retarded in food-deprived animals (Table 22 and Figure 24).

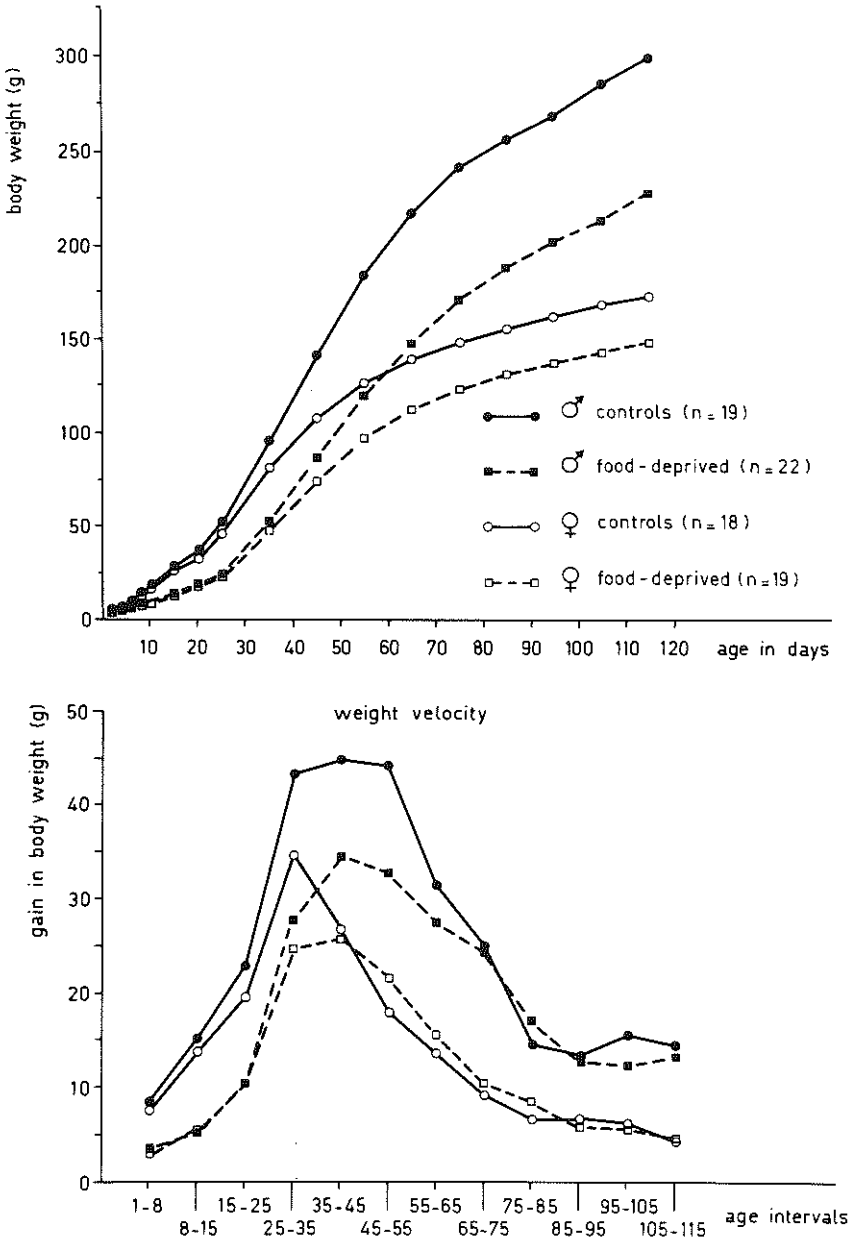



*Figure 21. Foster mother food deprivation experiment. Weight and velocity of body weight growth in male and female rats.*

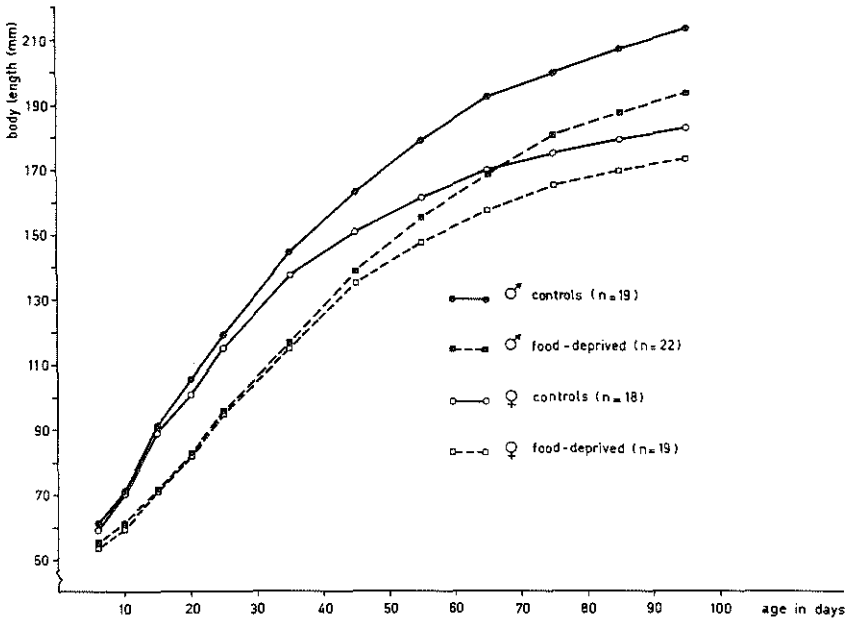



*Figure 22. Foster mother food deprivation experiment. Body length in male and female rats.*

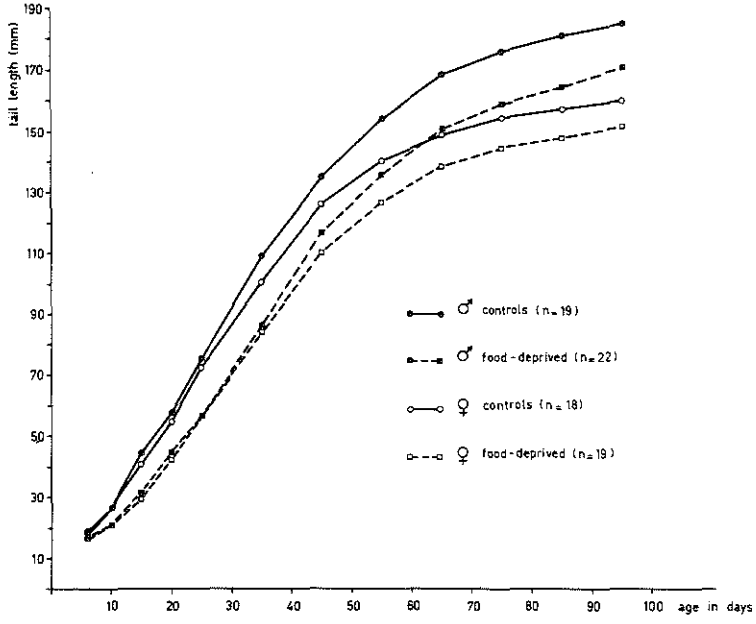



*Figure 23. Foster mother food deprivation experiment. Tail length in male and female rats.*

| | | N | Both eyes open | Auditory reflex | Landing reflex |
|---|---|---|---|---|---|
| Males | Foster mother food-deprived | 30 | 15.2$^{+}$ | 14.1$^{+}$ | 17.3$^{+}$ |
| | Control | 26 | 14.2 | 12.8 | 15.2 |
| Females | Foster mother food-deprivation | 27 | 15.4$^{+}$ | 14.2$^{++}$ | 17.7$^{+}$ |
| | Control | 25 | 14.2 | 13.1 | 15.4 |

For statistical analysis food-deprived animals were always compared with controls of the same sex using median tests ($\chi^2$).

$^{+}$ $p < .001$

$^{++}$ $p < .02$

*Table 22. Foster mother food deprivation experiment. Median ages in days at eye opening and at first occurrence of auditory and landing reflexes.*

*Skeletal maturity*

The bones of food-deprived animals were significantly less mature on Day 20 and significantly shorter on Days 20 and 60 (see Table 23). No difference was apparent between the Day and Night groups in any measure of physical development; they have therefore been combined in the presentation of the data.

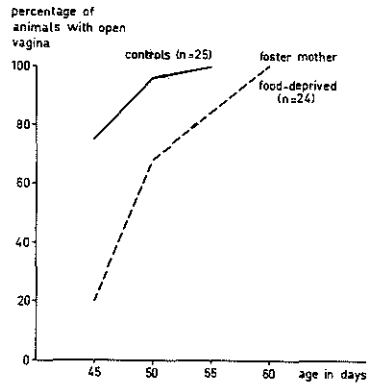



*Figure 24. Foster mother food deprivation experiment. Age at opening of the vagina.*

| | | N | Skeletal maturity score | | Length of radius (mm) | |
|---|---|---|---|---|---|---|
| | | | 20 days | 60 days | 20 days | 60 days |
| Males | Foster mother food-deprived | 25 | 40.9 | 57.2 | 11.0 | 20.3 |
| | Control | 15 | 44.8 | 58.1 | 13.4 | 22.7 |
| | | | U=26.5 p<.002 | ns | U=0 p<.002 | U=28.5 p<.002 |
| Females | Foster mother food-deprived | 22 | 41.1 | 56.6 | 10.8 | 19.4 |
| | Control | 16 | 45.4 | 57.8 | 13.1 | 20.7 |
| | | | U=5 p<.002 | ns | U=0 p<.002 | U=26 p<.002 |

*Table 23. Foster mother food deprivation experiment. Skeletal maturity and length of radius at two different ages.*

*Autopsy data*

The results of autopsy performed at 580 days of age are shown in tables 24, 25 and 26. The food deprivation regimen produced animals which never caught up in body weight, body length, and tail length with

| Treatment | N | Sex | Body weight (g) | Body length (mm) | Tail length (mm) |
|---|---|---|---|---|---|
| Food-deprived | 16 | ♂ | 348.5 | 224.2 | 195.2 |
| Control | 12 | ♂ | 425.5 | 238.2 | 210.1 |
| | | | U=12 P < .002 | U=14.5 P < .002 | U=2.5 P < .001 |
| Food-deprived (ovarex at 120 days of age) | 7 | ♀ | 230.3 | 196.7 | 174.6 |
| Food-deprived (intact) | 8 | ♀ | 209.4 | 193.8 | 171.4 |
| | | | U=6 p=.01 | U=16.5 ns | U=12 ns |
| Control (ovarex at 120 days of age) | 8 | ♀ | 287.2 | 206.8 | 182.1 |
| Control (intact) | 8 | ♀ | 267.1 | 205.1 | 180.9 |
| | | | U=17 ns | U=13.5 ns | U=27 ns |
| Statistics: | | | | | |
| $FD_{ovarex}$ vs $C_{ovarex}$ | | | U=0 p .001 | U=2 p=.002 | U=3 p=.002 |
| $FD_{intact}$ vs $C_{intact}$ | | | U=1 P < .001 | U=0 P < .001 | U=2 P < .001 |

*Table 24. Foster mother food deprivation experiment. Weight and measurements at 580 days of age.*

their well-fed controls. At the age of 580 days food-deprived males weighed 82% of the control body weight, and measured 94% of the control body length and 93% of the control tail length. For the intact food-deprived females these percentages were 78%, 94% and 91% respectively. As can be seen in table 24 ovariectomy in adulthood (at 120 days of age) caused a significantly higher body weight for the food-deprived females ($U = 6$, $p < .01$), whereas body weight of control females did not differ significantly ($U = 17$, n.s.). Body length and tail length were not significantly affected by ovariectomy in adulthood.

The absolute and relative weights of some organs are given in tables 25 and 26. The food deprivation regimen produced animals with lighter (and presumably smaller) brains (males, $U = 10$, $p < .04$; females, $U = 0$, $p < .056$), but relative to body weight their brains were heavier than those of the control animals (males, $U = 0$, $p < .001$; females, $U = 0$, $p < .056$). This clearly demonstrates the well established brain sparing phenomenon occurring with undernutrition (Dobbing, 1970). Furthermore, only the absolute adrenal and testes weights were significantly lower in the food-deprived males, but the weights were normal relative to body weight.

| Treatment | N | Sex | Body weight (g) | Brain (mg) | Thyroid (mg) | Adrenals (mg) | Seminal vesicles Uterus (mg) | Testes (mg) |
|---|---|---|---|---|---|---|---|---|
| Food-deprived | 8 | ♂ | 324.9 | 2117 | 26.7 | 45.2 | 196.0 | 2231 |
| Control | 6 | ♂ | 415.5 | 2291 | 28.8 | 51.8 | 211.7 | 2599 |
| | | | U=0 p < .001 | U=10 p=.04 | U=15.5 ns | U=6 p=.01 | U=19 ns | U=9 p=.02 |
| Food-deprived (ovarex at 120 days of age) | 3 | ♀ | 218.7 | 1925 | 22.1 | 44.9 | 77.9 | |
| Control (ovarex at 120 days of age) | 4 | ♀ | 288.8 | 2052 | 23.2 | 49.5 | 84.4 | |
| | | | U=0 p=.056 | U=0 p=.056 | U=3 ns | U=3 ns | U=6 ns | |

*Table 25. Foster mother food deprivation experiment. Body and organ weights at 580 days of age.*

| Treatment | N | Sex | Brain | Thyroid | Adrenals | Seminal vesicles Uterus | Testes |
|---|---|---|---|---|---|---|---|
| Food-deprived | 8 | ♂ | 683 | 8.2 | 13.9 | 60.5 | 688 |
| Control | 6 | ♂ | 551 | 6.9 | 12.5 | 51.0 | 625 |
| | | | U=0 $p < .001$ | U=11.5 ns | U=11.5 ns | U=9 p=.06 | U=12 ns |
| Food-deprived (ovarex at 120 days of age) | 3 | ♀ | 881 | 10.1 | 20.5 | 35.7 | |
| Control (ovarex at 120 days of age) | 4 | ♀ | 714 | 8.1 | 17.2 | 29.4 | |
| | | | U=0 p=.056 | U=0 p=.056 | U=1 ns | U=1 ns | |

*Table 26. Foster mother food deprivation experiment. Organ weights in mg/100 g body weight at 580 days of age.*

Behavioural measures

*Open field*

Fifty-seven animals (18 food-deprived males, 11 control males, 16 food-deprived females, and 12 control females) were tested individually in an open field at the age of 75 days. As can be seen from table 27, there were no differences between experimental and control animals for either sex, but females displayed significantly more exploratory behaviour than males ($t$ = 2.45, $df$ = 55, $p$ <.02).

*Elevated-runway test*

In view of the evidence that early malnutrition in the rat has its most serious effect in the brain on cerebellar development (Culley and Lineberger, 1968; Howard and Granoff, 1968; Chase, Lindsley and O'Brien, 1969; Fish and Winick, 1969; Dobbing, 1971a; Smart, 1971; Altman and McCrady, 1972), a test of balance and motor coordination was devised. The animals were trained to walk from a platform over a runway (length 80 cm) to another platform for food reward (see Figure 25). After each animal had completed 10 successive runs under 10 sec each, the 8 cm wide training runway was replaced by successively narrower runways. The time

| | | N | Squares entered | Boluses produced |
|---|---|---|---|---|
| Males | Foster mother food-deprived | 18 | 122.0 | 1.0 |
| | Control | 11 | 122.3 | 1.8 |
| | All males | 29 | 122.1 | 1.3 |
| Females | Foster mother food-deprived | 16 | 154.2 | 1.0 |
| | Control | 12 | 148.3 | .4 |
| | All females | 28 | 151.7+ | .8 |

+ Significantly different from males ($t = 2.45$, $p < .02$)

*Table 27. Foster mother food deprivation experiment. Mean number of squares entered and of boluses produced in the open field test.*

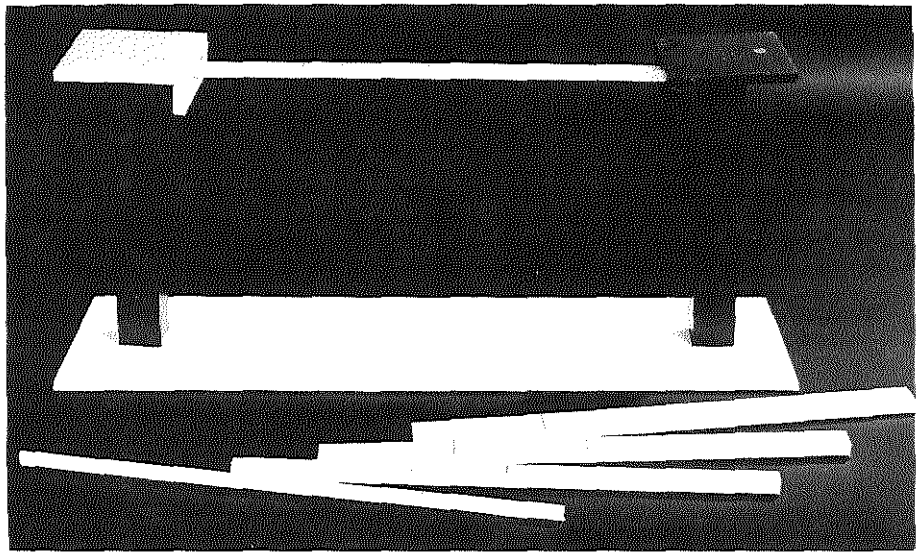


*Figure 25. Foster mother food deprivation experiment. The elevated-runway apparatus used in testing balance and motor co-ordination.*

taken to cross the runway and the number of slips, falls, and refusals were recorded during 5 trials for each test runway.

Thirteen males (8 food-deprived and 5 control) and 13 females (8 food-deprived and 5 control) were tested between 80 and 100 days of age. There were no consistent differences between the groups in refusals, slips, falls, or time taken to cross the runway (see Table 28). Thus, as judged by this test, there is no support for the hypothesis that food-deprived animals show poorer balance and coordination.

*Residential plus maze*

Twenty-six males (15 food-deprived and 11 control) were placed in the plus maze for periods of 8 days each between the ages of 110 and 150 days. As can be seen from figure 26, the food-deprived males were more active than the controls ($t = 3.78$, $df = 24$, $p < .001$).

Twelve females (6 food-deprived and 6 control) were also tested between Days 110 and 150, for a period of 9 days each. The food-deprived females were more active than the controls ($t = 5.6$, $df = 10$, $p < .05$). Each group of females was also more active than the corresponding group of males (food-deprived animals, $t = 4.76$, $df = 19$, $p < .001$; control animals, $t = 3.56$, $df = 15$, $p < .01$), but control females were not more

| | | N | Width of the runway (cm.) | | | |
|---|---|---|---|---|---|---|
| | | | 8 | 3 | 2 | 1 |
| Males | Foster mother food-deprived | 8 | 2.4 | 4.1 (1R) | 4.2 (1R) | 7.6 (3R) |
| | Controls | 5 | 3.0 | 4.0 (1R) | 4.7 (1R) | 7.0 (2R) |
| Females | Foster mother food-deprived | 8 | 2.2 | 4.5 (2R) | 6.5 (1R) | 5.3 (2R) |
| | Controls | 5 | 3.0 | 5.2 | 6.3 | 8.4 (1R) |

The number of animals that refused to cros the runway within 3 minutes is shown in parentheses. The mean given is based only on those animals that crossed the runway.

*Table 28. Foster mother food deprivation experiment. Mean running time in seconds in the elevated-runway test.*

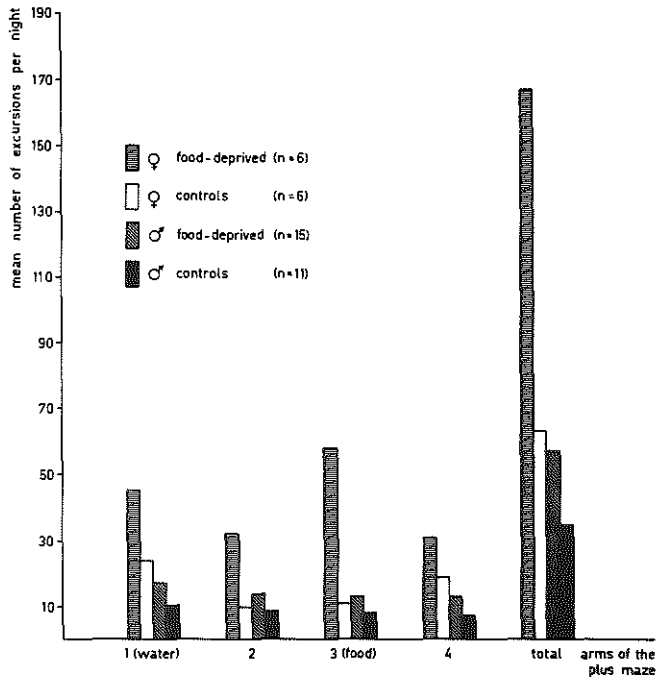



*Figure 26. Foster mother food deprivation experiment. Mean number of excursions per night (21.00 to 07.00 hours) into each of the arms of the plus maze.*

active than food-deprived males ($t$ = .63, $df$ = 19, n.s.). This higher activity level in the females might have been caused by the occurrence of behavioural oestrus (e.g. Slonaker, 1924; Finger, 1969; Wade, 1972). In order to test this possibility, the females were ovariectomized and placed in the plus maze again 2 weeks later. Activity was considerably less during this post-ovariectomy test period (Figure 27; $F$ = 16.6, $df$ = 1/10, $p$ <.01), and was no longer different from that of the males ($t$'s $\leq$1.6, n.s.). Results from another litter of females tested twice after ovariectomy indicate that the decrease after ovariectomy may be partially due to habituation to the apparatus rather than to the effects of ovariectomy alone.

In no case was a significant preference for the food arm of the plus maze observed in either the food-deprived or the control animals.

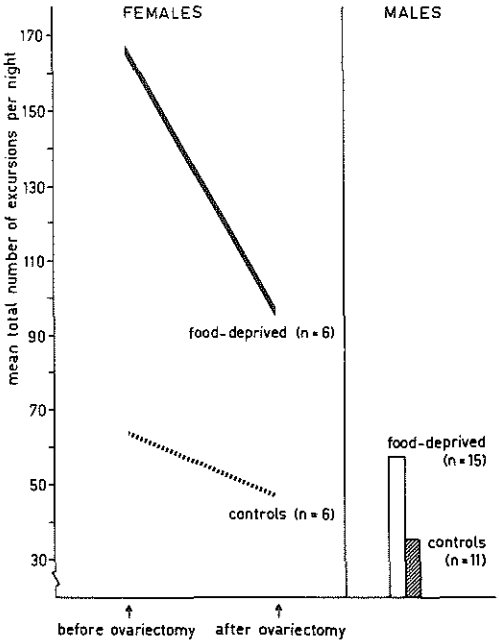



*Figure 27. Foster mother food deprivation experiment. Mean number of excursions per night into all arms of the plus maze for food-deprived and control females before and after ovariectomy, and for food-deprived and control males.*

*Hebb-Williams maze*

Eleven males (7 food-deprived and 4 control) and 9 ovariectomized females (5 food-deprived and 4 control) were tested in the Hebb-Williams maze between the ages of 130 and 170 days.

Whereas some previous investigations have yielded evidence of poorer performance by food-deprived animals in this test (Cowley and Griesel, 1959, 1963, 1969; Ottinger and Tanabe, 1968), no significant differences in error scores between food-deprived and control animals were observed in the present study (Table 29). On the contrary, the food-deprived animals made fewer errors than the controls, which corroborates the findings of Smart (1971). Furthermore, food-deprived males ran significantly faster than control males ($t$ = 2.58, $df$ = 9, $p$ <.05). Mean running time per trial was 9.7 sec for the experimental males and 13.2 sec for the control males. Food-deprived females also ran faster than

| | | N | Number of errors over 12 problems | Total time over 12 problems (sec) |
|---|---|---|---|---|
| Males | Foster mother Food-deprived | 7 | 164.0 | 939 |
| | Control | 4 | 172.2 | 1267 |
| Females | Foster mother Food-deprived | 5 | 181.6 | 880 |
| | Control | 4 | 194.2 | 935 |

Significantly different from control males ($t = 2.58$, $p < .05$)

*Table 29. Foster mother food deprivation experiment. Mean error scores and running times in the Hebb-Williams maze.*

control females, but not significantly so.

*Thompson box*

Nine males (6 food-deprived and 3 control) and 8 ovariectomized females (4 food-deprived and 4 control) were taught 2 visual discrimination problems in the shock-motivated Thompson box between the ages of 230 and 245 days. As soon as each animal had reached the criterion in the black-white squares discrimination, the animal was taught a horizontal-vertical bar discrimination. As is shown in table 30, there were no differences between food-deprived and control animals in the number of trials required to reach criterion for either problem. Running times were always shorter for the food-deprived animals than for their controls. The difference was significant in males for the black-white problem ($t = 2.37$, $df = 7$, $p < .05$) and approached significance in females for both problems (black-white, $t = 1.6$, $df = 6$, $p < .10$; horizontal-vertical, $t = 1.8$, $df = 6$, $p < .10$).

| | | | Discrimination problem | | | |
|---|---|---|---|---|---|---|
| | | | Black - White | | Horizontal - Vertical bars | |
| | | N | Trials to criterion | Running[(a)] time (sec) | Trials to criterion | Running[(a)] time (sec) |
| Males | Foster mother food-deprived | 6 | 120 | 4.7[+] | 96.7 | 9.4 |
| | Control | 3 | 120 | 7.9 | 120 | 10.2 |
| Females | Foster mother food-deprived | 4 | 150 | 5.1 | 125 | 11.5 |
| | Control | 4 | 115 | 8.0 | 110 | 18.1 |

(a) in the series during which the animal reached criterion

[+] Significantly different from control males ($t = 2.37$, $p < .05$)

*Table 30. Foster mother food deprivation experiment. Mean number of trials to criterion and mean running times in the Thompson box.*

*Fertility and pup retrieval*

Fourteen females (7 food-deprived and 7 control) were tested for fertility at the age of approximately 220 days. One male was placed in a cage housing 3 or 4 females for 14 days; after this period the females were housed individually. All females became pregnant, with the exception of 1 control animal. Following a normal gestation period of about 21 days, 6 food-deprived (1 animal died just before delivery) and 6 controls gave birth to litters with an average number of 6.2 and 7.5 pups respectively (see Table 31). There were no differences in neonatal mortality and 2 days after delivery the average litter sizes were still not significantly different.

Eleven females (5 food-deprived and 6 control) were subsequently tested twice for maternal behaviour. In this test all pups were taken out of the nest and the mother was left undisturbed for 5 minutes. After this period 5 pups (2 of her own and 3 of another litter) were placed in the cage in a corner opposite to the nest and the number of pups retrieved during a 10 minutes period was counted. As can be seen

| | N | Litter Size | |
|---|---|---|---|
| | | at Delivery | 2 Days after Delivery |
| Food-deprived | 6 | 6.2 | 4.7 |
| Control | 6 | 7.5 | 6.7 |
| | | U=13.5 | U=11.5 |
| | | ns | ns |

*Table 31. Foster mother food deprivation experiment. Mean sizes of litters produced by experimental and control animals.*

| | N | Number of Pups Retrieved | |
|---|---|---|---|
| | | 5-6 Days after Delivery | 10-12 Days after Delivery |
| Food-deprived | 5 | 2.4 | 2.0 |
| Control | 6 | 3.5 | 2.5 |
| | | U=19 | U=13 |
| | | ns | ns |

*Table 32. Foster mother food deprivation experiment. Mean number of young retrieved in "pup retrieval test" by experimental and control animals.*

from table 32 there were no differences between food-deprived and control females, neither when the young were 5 - 6 days of age, nor when they were 10 - 12 days old. Two of the food-deprived and 1 of the control females never retrieved any pups in either test.

*Mating*

It has been shown that male rats reared in litters of 12 displayed less copulatory behaviour in adulthood compared to males reared in litters of 6 (Seitz, 1954). In other experiments tube-fed incubator-reared male rats have been found not to copulate and ejaculate in mating

tests carried out at 200 days of age (Gruendel and Arnold, 1969). However, animals reared in large litters or in an incubator without maternal feeding, become undernourished. The observed deviations in adult copulatory behaviour may, therefore, have been caused by undernutrition, rather than by social or maternal deprivation.

In order to test this hypothesis 13 males (8 food-deprived and 5 control) were tested for mating behaviour at 300 days of age. Each male was tested once with two receptive females simultaneously in a semi-circular test cage with a glass front and a grid floor. Ovariectomized females were made sexually receptive by a single injection of oestradiol benzoate (10 γ/.1 ml oil), followed 28 hours later by a single injection of progesterone (.5 mg/.1 ml oil). The females were placed with the male 5 hours after the progesterone injection. One experimenter observed the male rats and the copulatory behaviours were scored. The test was terminated following ejaculation or after 15 minutes if no ejaculation had occurred by that time. As is shown in table 33 there were no differences between the food-deprived and the control males in any of the scored copulatory behaviours. Thus we may conclude that in this experiment undernutrition early in life had not affected adult mating behaviour in the male rat.

| Treatment | N | Mounts | Mounts with thrusts | Intromissions | Ejaculation |
|---|---|---|---|---|---|
| Food-deprived | 8 | 5.5 | 34.2 | 7.1 | .6 |
| Control | 5 | 5.2 | 33.2 | 6.4 | .6 |
| | | U=18 | U=20 | U=22.5 | |
| | | ns | ns | ns | |

*Table 33. Foster mother food deprivation experiment. Mean scores of male copulatory behaviours during a 15 minutes' test.*

## CONCLUSIONS

Early undernutrition in the rat *per se* (a) does not affect exploratory behaviour, emotionality, and learning, and (b) causes a permanent increase in locomotor activity.

# EXPERIMENT 2. INCUBATOR FOOD DEPRIVATION

## METHODS

### Deprivation regimen

In general the same procedure was followed as that described for the foster mother food deprivation experiment, with a few exceptions. Pups from 8 litters were combined to constitute 3 mixed litters. The animals were food deprived for 12 hours per day by placement in an incubator with a relative humidity of 70 - 80% and a constant temperature of 29°C. During the light period the inside of the incubator was illuminated with a 60 Watt light bulb. Again litters were kept at a standard size of 8 pups at all times. Six food-deprived and 6 control females were ovariectomized at 115 days of age.

### Measurement of physical development

The rats were weighed and measured between 09.00 and 10.00 hours. The animals were weighed every 5th day until Day 25, and thereafter every 10th day until Day 115. Body length and tail length were measured on Days 10, 20, 25, 45, 95, and 115.

Beginning on Day 11 all animals were checked daily for eye opening, and from Day 35 females were daily checked for vaginal opening.

### Behavioural measures

In adulthood the animals were subjected to various tests (see General Introduction, Table 1). The tests were administered in the same order for experimental and control animals; food-deprived and control animals of either sex were always tested at the same time.

# RESULTS

## Physical measures

*Somatic growth*

The data are shown in figures 28 and 29. At the age of 115 days the food-deprived animals, compared to their controls, were significantly lighter (males, U = 2, $p$ <.002; females, U = 5, $p$ <.02) with shorter bodies (males, U = 0, $p$ <.002; females, U = 3, $p$ <.003) and tails (males, U = 9, $p$ <.02; females, U = 11.5, $p$ <.05).

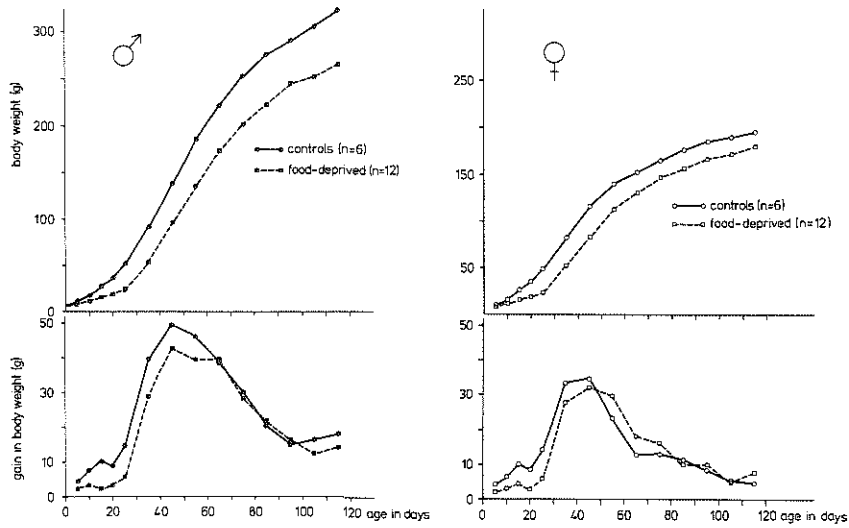



*Figure 28. Incubator food deprivation experiment. Weight and velocity of body weight growth in male and female rats.*

Eye opening was not significantly retarded in food-deprived animals (mean age 13.5 days for control and 14 days for food-deprived rats of either sex). In the food-deprived females the average age at vaginal opening was significantly raised (almost 4 days), while the average body weight at the time of opening of the vagina was 80%, in comparison with controls (see Table 34).

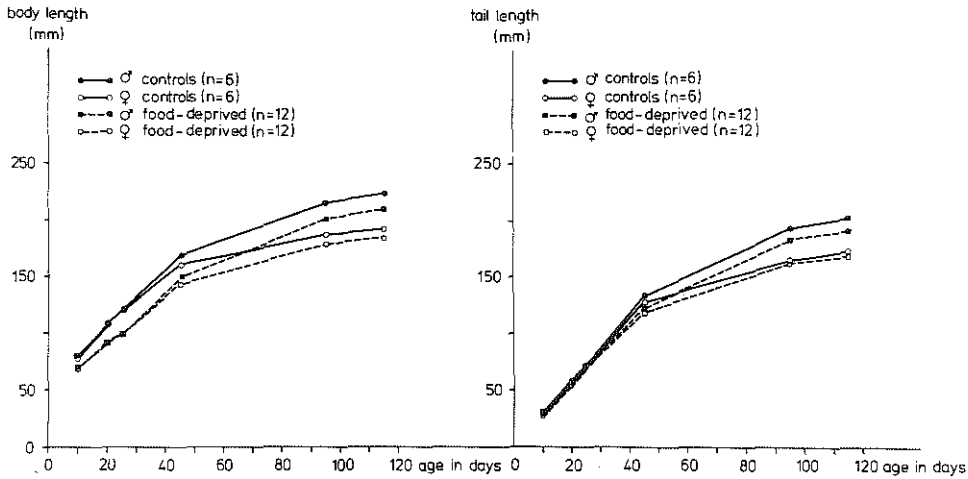



*Figure 29. Incubator food deprivation experiment. Body length and tail length in male and female rats.*

| | N | Age (days) | Body weight (g) |
|---|---|---|---|
| Incubator food-deprived | 12 | 44.5$^{+}$ | 82.2$^{++}$ |
| Control | 6 | 40.8 | 102.5 |

$^{+}$ significantly different from controls ($t=3.38$, $p<.01$)

$^{++}$ significantly different from controls ($t=5.89$, $p<.001$)

*Table 34. Incubator food deprivation experiment. Mean age and body weight at vaginal opening.*

*Autopsy data*

Autopsy was performed at 260 days of age; the results are shown in tables 35 and 36. As in the foster mother food deprivation experiment, there was no catch up growth in body weight, body length, or tail length in incubator food-deprived animals of either sex. At the age of 260 days the food-deprived males weighed and measured 82%, 95%, and 94% of the control body weight, body length, and tail length respectively. For

| Treatment | N | Sex | Body weight (g) | Body length (mm) | Tail length (mm) | Brain (mg) | Thyroid (mg) | Adrenals (mg) | Seminal vesicles or Uterus (mg) | Testes or Ovaries (mg) |
|---|---|---|---|---|---|---|---|---|---|---|
| Food-deprived | 12 | ♂ | 319.8 | 222.1 | 202.2 | 1870 | 26.5 | 37.6 | 303.8 | 2404 |
| Control | 6 | ♂ | 389.7 | 233.7 | 215.0 | 2080 | 30.4 | 44.0 | 394.0 | 2942 |
| | | | U=4 p=.002 | U=4 p=.002 | U=8 p < .02 | U=6 p < .02 | U=25 ns | U=12 p < .05 | U=1 p < .002 | U=0 p < .002 |
| Food-deprived (intact) | 6 | ♀ | 212.3 | 192.2 | 175.7 | 1789 | 21.1+ | 48.5+ | 473.0 | 59.9 |
| Food-deprived (ovarex at 115 days of age) | 6 | ♀ | 228.2 | 196.8 | 179.3 | 1799 | 20.3 | 47.0 | 105.4 | |
| | | | U=8 ns | U=5.5 p < .05 | U=8 ns | U=16 ns | U=18 ns | U=10 ns | | |
| Control (ovarex at 115 days of age) | 6 | ♀ | 270.3 | 205.5 | 184.3 | 1941 | 21.8+ | 53.2 | 111.8 | |
| Statistics: $FD_{ovarex}$ vs $C_{ovarex}$ | | | U=0 p=.002 | U=0 p=.002 | U=7 p=.047 (one tailed) | U=4.5 p=.034 | U=18 ns | U=8 ns | U=12 ns | |

+ n=5

*Table 35. Incubator food deprivation experiment. Measurements and weights at 260 days of age.*

the ovariectomized females these percentages were 84, 96, and 97.

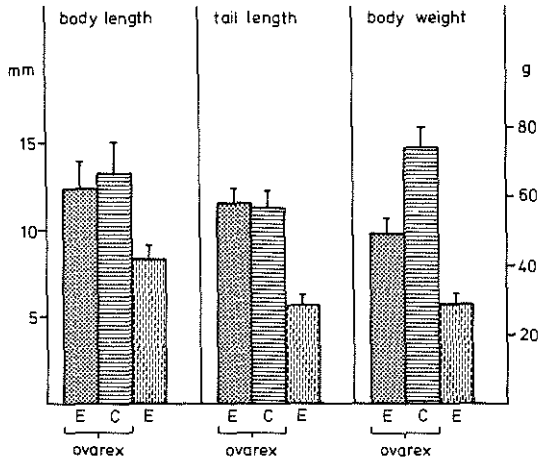



*Figure 30. Incubator food deprivation experiment. Increments (means + S.E.) in body length, tail length and body weight over a 145 day period in intact and ovariectomized experimental (E) and control (C) animals. Ovariectomies were done at 115 days of age. Each group consisted of 6 animals.*

As can be seen in figure 30 ovariectomy at 115 days of age caused a significant increase in body weight, body length, and tail length in the food-deprived females (body weight, $U = 3$, $p < .02$; body length, $U = 4$, $p = .026$; tail length, $U = 0$, $p = .002$). Since they had been all ovariectomized the effects of ovariectomy could not be studied in the control females. Comparisons between ovariectomized experimental and control females revealed no significant differences in body length and tail length increments (U's = 18 and 17 respectively, n.s.). However, control females showed a significantly bigger increase in body weight than (in response to ovariectomy) food-deprived females ($U = 4.5$, $p < .05$).

The food deprivation regimen produced animals with lighter brains (males, $U = 6$, $p < .02$; females, $U = 4.5$, $p = .034$). Relative to body weight food-deprived females had heavier brains than control females ($U = 3$, $p = .016$); this was also the case for the males but not signifi-

| Treatment | N | Sex | Brain | Thyroid | Adrenals | Seminal vesicles or Uterus | Testes or Ovaries |
|---|---|---|---|---|---|---|---|
| Food-deprived | 12 | ♂ | 592 | 8.3 | 11.8 | 94.9 | 759 |
| Control | 6 | ♂ | 538 | 7.8 | 11.2 | 101.4 | 756 |
| | | | U=22 | U=27.5 | U=26 | U=17 | U=33.5 |
| | | | ns | ns | ns | ns | ns |
| Food-deprived (intact) | 6 | ♀ | 846 | 10.0+ | 22.8+ | 223.8 | 28.3 |
| Food-deprived (ovarex at 115 days of age) | 6 | ♀ | 791 | 8.9 | 20.6 | 46.6 | |
| | | | U=9 | U=12 | U=8.5 | | |
| | | | ns | ns | ns | | |
| Control (ovarex at 115 days of age) | 6 | ♀ | 723 | 8.0+ | 19.6 | 41.9 | |
| Statistics: | | | | | | | |
| $FD_{ovarex}$ vs $C_{ovarex}$ | | | U=3 | U=6 | U=14 | U=14 | |
| | | | p=.016 | ns | ns | ns | |

+ n=5

*Table 36. Incubator food deprivation experiment. Organ weights in mg/100 g body weight at 260 days of age.*

cantly so (U = 22, n.s.). Furthermore, the food-deprived males had significantly lower adrenal, seminal vesicle, and testis weights than control males, but relative to body weight there were no differences. In the females the food deprivation regimen did not produce any significant differences in thyroid, adrenal, and uterus weights; note that comparisons are only possible for ovariectomized females.

## Behavioural measures

### *Open field*

All animals (12 food-deprived males, 6 control males, 12 food-deprived females, 6 control females) were tested individually in an open field apparatus at the age of 90 days. As can be seen from table 37 there were no differences in ambulation and defaecation between experimental and control animals for either sex. Males displayed significantly less

| | | N | Squares entered | Boluses produced |
|---|---|---|---|---|
| Males | Incubator food-deprived | 12 | 105.7 | 1.4 |
| | Control | 6 | 108.2 | 3.8 |
| | All males | 18 | 106.5+ | 2.2 |
| Females | Incubator food-deprived | 12 | 155.2 | 1.1 |
| | Control | 6 | 168.0 | 1.0 |
| | All females | 18 | 159.4 | 1.1 |

+ significantly different from "all females" ($t$=5.79, $p<.001$)

*Table 37. Incubator food deprivation experiment. Mean number of squares entered and of boluses produced in the open field test.*

exploratory behaviour than females ($t$ = 5.79, $df$ = 34, $p$ <.001).

*Hebb-Williams maze*

Twelve males (6 food-deprived and 6 control) and 12 ovariectomized females (6 food-deprived and 6 control) were tested in the Hebb-Williams maze between the ages of 120 and 150 days. No significant differences in error scores and running times were observed between food-deprived and control animals of either sex (Table 38).

| | | N | Number of errors over 12 problems | Total time over 12 problems (sec) |
|---|---|---|---|---|
| Males | Incubator food-deprived | 6 | 157.0 | 635 |
| | Control | 6 | 149.2 | 619 |
| Females | Incubator food-deprived | 6 | 169.0 | 610 |
| | Control | 6 | 167.3 | 674 |

*Table 38. Incubator food deprivation experiment. Mean error scores and running times in the Hebb-Williams maze.*

*Residential plus maze*

Twelve males (6 food-deprived and 6 control) and 12 ovariectomized females (6 food-deprived and 6 control) were placed in the plus maze for periods of 9 days each between the ages of 200 and 230 days. As can be seen from figure 31, the food-deprived males were more active than their controls (U = 3, $p$ = .03). Food-deprived females were less active than

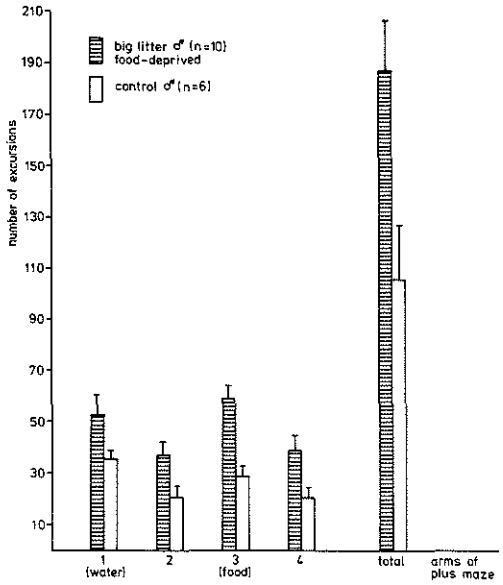



*Figure 31. Incubator food deprivation experiment. Total number of excursions over 4 nights (21.00 to 09.00 hours) into each of the arms of the plus maze (means + S.E.).*

controls but not significantly so (U = 10, n.s.). However, food-deprived females were significantly less active than food-deprived males (U = 2, $p$ = .032), but displayed the same activity as control males. Control females did not differ significantly from either experimental or control males.

Food-deprived females and both groups of males showed a preference

for the food and water arms of the plus maze; control females did not show significant preferences for any of the plus maze arms.

During their stay in the plus maze the animals did not lose weight and the daily food intake was fairly constant per animal. Although the initial body weights of food-deprived and control animals were sgnificantly different (males, $U = 2$, $p = .008$; females, $U = 0$, $p = .002$), the mean daily food intake did not differ significantly between experimental and control animals (see Table 39).

| | | N | Food intake per day (g) | Body weight (g) |
|---|---|---|---|---|
| Males | Incubator food-deprived | 6 | 13.4 | $323.5^{+}$ |
| | Control | 6 | 14.6 | 392.3 |
| Females | Incubator food-deprived | 6 | 10.6 | $231.8^{++}$ |
| | Control | 6 | 11.4 | 269.2 |

$^{+}$ significantly different from control males (U=2, p= .008)

$^{++}$ significantly different from control females (U=0, p= .002)

*Table 39. Incubator food deprivation experiment. Mean daily food intake during 9 days in the residential plus maze and initial mean body weight.*

*Thompson box*

Finally the same animals which had been subjected to the previous tests were taught 1 visual discrimination problem (horizontal-vertical bars) in the shock-motivated Thompson box between the ages of 240 and 260 days. As is shown in table 40 the food-deprived animals required significantly more trials to meet the criterion than controls (males, $U = 7.5$, $p = .056$ one-tailed; females, $U = 2$, $p = .008$). There were no significant differences in running time between experimental and control animals, but males ran faster than females ( $U = 27$, $p < .02$).

| | | N | Trials to criterion | Running time[a] (sec) |
|---|---|---|---|---|
| Males | Incubator food-deprived | 6 | 166.7+ | 3.3 |
| | Control | 6 | 126.7 | 2.2 |
| | All males | 12 | -- | 2.8" |
| Females | Incubator food-deprived | 6 | 176.7++ | 5.2 |
| | Control | 6 | 126.7 | 5.6 |
| | All females | 12 | -- | 5.4 |

(a) in the series during which the animal reached criterion

\+ significantly different from control males (U=7.5, p= .056 one tailed)

++ significantly different from control females (U=2, p= .008)

" significantly different from "all females" (U=27, $p < .02$)

*Table 40. Incubator food deprivation experiment. Mean number of trials to criterion and mean running times in the Thompson box.*

## CONCLUSION

Early undernutrition combined with restricted maternal care impairs shock-motivated learning in the rat.

# EXPERIMENT 3. BIG LITTER FOOD DEPRIVATION

## METHODS

### Deprivation regimen

Twenty female rats were placed with males simultaneously in order to ensure that several litters be born on the same day. Male pups of 14 litters were weighed and marked with coloured ink on the day of birth (Day 1). On Day 2, male pups from 4 or 5 litters were combined to constitute one big litter of 18 pups. In all, 3 big litters and 1 litter of 8 pups were formed.

Mortality in the big litters was about 15% but in the control litter 2 animals died at the age of 14 days. In order to keep the litter size constant in the control litter, the dead animals were replaced by pups of the same age, and left in the litter till the time of weaning.

### Measurement of physical development

As in experiments 1 and 2, the rats were weighed and measured between 09.00 and 10.00 hours. Weighing of the animals occurred every 5th day until Day 25, and thereafter every 10th day until Day 115. Body length and tail length were measured on Days 10, 20, 25, 45, 85, and 115 (at 115 days one big litter was not measured).

### Behavioural measures

In adulthood subgroups of the food-deprived animals and all controls were subjected to various tests (see General Introduction, Table 1). As has been stated before, the tests were administered at the same time for the food-deprived and control animals.

# RESULTS

## Physical measures

*Somatic growth*

The data are shown in figures 32 and 33. At 115 days of age the experimental males were significantly lighter than the controls ($U = 63$, $z = -2.44$, $p = .014$). However, there were no significant differences in body length ($U = 59$, $z = -1.40$, n.s.) and tail length ($U = 64.5$, $z = -1.17$, n.s.).

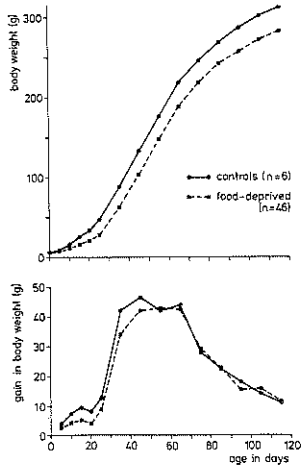



*Figure 32. Big litter food deprivation experiment. Weight and velocity of body weight growth in male rats.*

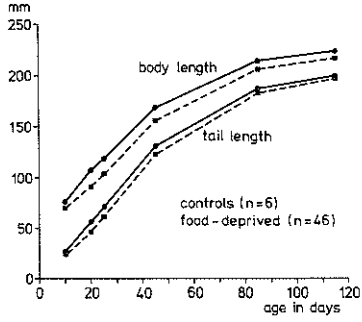



*Figure 33. Big litter food deprivation experiment. Body length and tail length in male rats.*

*Autopsy data*

Autopsy was performed at 250 days of age. Only data on the animals subjected to behaviour tests are shown in tables 41 and 42. In contrast with those of the previous two experiments, the big litter food-deprived males showed catch up growth in body weight. At the age of 250 days the food-deprived males weighed 91% of the control body weight, and the difference in mean body weight was only "borderline" significant (U = 14, $p$ = .05 one-tailed).

| Treatment | N | Body weight (g) | Body length (mm) | Tail length (mm) | Brain (mg) | Thyroid (mg) | Adrenals (mg) | Testes (mg) |
|---|---|---|---|---|---|---|---|---|
| Food deprived | 10 | 367.5 | 235.6 | 207.3 | 1950 | 26.5 | 30.6 | 2540 |
| Control | 6 | 402.2 | 243.5 | 210.7 | 2146 | 28.8 | 39.0 | 2852 |
| | | U=14 | U=18.5 | U=22 | U=9 | U=23 | U=3.5 | U=8 |
| | | p=.05 (one tailed) | ns | ns | p < .05 | ns | p < .02 | p=.02 |

*Table 41. Big litter food deprivation experiment. Measurements and weights at 250 days of age.*

The food deprivation regimen produced animals with lighter brains (U = 9, $p$ <.05), lighter adrenals (U = 3.5, $p$ <.02), and lighter testes (U = 8, $p$ = .02). Relative to body weight only the adrenals weighed significantly less than those of the controls (U = 10, $p$ <.05).

| | N | Brain | Thyroid | Adrenals | Testes |
|---|---|---|---|---|---|
| Food-deprived | 10 | 532 | 7.2 | 8.4 | 695 |
| Control | 6 | 536 | 7.2 | 9.7 | 713 |
| | | U=23 | U=25 | U=10 | U=28.5 |
| | | ns | ns | p < .05 | ns |

*Table 42. Big litter food deprivation experiment. Organ weights in mg/100 g body weight at 250 days of age.*

Behavioural measures

*Open field*

Twenty-four animals (18 food-deprived and 6 control) were tested individually in an open field at 90 days of age. As can be seen from table 43 the food-deprived males displayed significantly more explora-

| | N | Squares entered | Boluses produced |
|---|---|---|---|
| Big litter food-deprived | 18 | 153.8$^{+}$ | 1.9 |
| Control | 6 | 128.7 | 1.7 |

$^{+}$ significantly different from control males (U=19.5, $p < .05$)

*Table 43. Big litter food deprivation experiment. Mean number of squares entered and of boluses produced in the open field test.*

tory behaviour than controls ($t$ = 2.42, $df$ = 22, $p$ <.05). Comparison of these data with those of the incubator food deprivation animals, showed that big litter food-deprived males displayed significantly more exploratory behaviour than incubator "all males" ($t$ = 5.63, $df$ = 34, $p$ <.001). Big litter control males did not show significantly more exploratory behaviour than incubator food-deprived males ($t$ = 1.72, $df$ = 22, n.s.), but scored significantly lower than incubator "all females" ($t$ = 2.53, $df$ = 22, $p$ <.02).

*Hebb-Williams maze*

Sixteen animals (10 food-deprived and 6 control) were tested in the Hebb-Williams maze between the ages of 140 and 175 days. As can be seen in table 44 the food-deprived males scored significantly fewer errors than the controls (U = 9.5, $p$ <.05). The experimental males also had shorter running times, but not significantly so (U = 20, n.s.).

*Residential plus maze*

The same animals which had been tested in the Hebb-Williams maze, were placed in the residential plus maze for periods of 9 days each

| | N | Number of errors over 12 problems | Total time over 12 problems (sec) |
|---|---|---|---|
| Big litter food-deprived | 10 | 149.5+ | 624 |
| Control | 6 | 161.8 | 751 |

+ significantly different from control males (U=9.5, $p < .05$)

*Table 44. Big litter food deprivation experiment. Mean error scores and running times in the Hebb-Williams maze.*

between the ages of 210 and 240 days. Figure 34 clearly shows that big litter food-deprived males were significantly more active than control males (U = 6, $p < .02$).

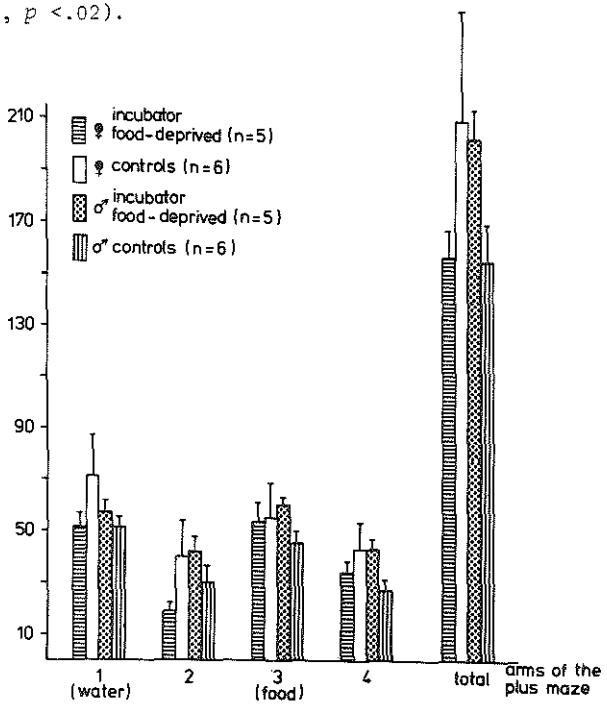



*Figure 34. Big litter food deprivation experiment. Total number of excursions over 4 nights (21.00 to 09.00 hours) into each of the arms of the plus maze (mean + S.E.).*

During their stay in the plus maze the animals did not lose weight and the individual daily food intake was rather constant. The initial body weights were borderline significantly different between food-deprived and control animals (U = 14, $p$ = .05 one-tailed), but there was no significant difference in the average daily food intake between experimental and control animals (see Table 45).

| | N | Food intake per day (g) | Body weight (g) |
|---|---|---|---|
| Big litter food-deprived | 10 | 13.9 | 355.6$^{+}$ |
| Control | 6 | 14.6 | 392.3 |

$^{+}$ significantly different from control males (U=14, p=.05 one tailed)

*Table 45. Big litter food deprivation experiment. Mean daily food intake during 9 days in the residential plus maze and mean initial body weight.*

## CONCLUSIONS

Rearing rats in big litters (15 - 16) cause (a) increased exploratory behaviour, (b) improved food-motivated learning, and (c) increased locomotor activity.

## DISCUSSION OF PART B

For clarity's sake the *foster mother* food deprivation experiment will be discussed first. This experiment has demonstrated that neonatally food-deprived male and female rats which have had permanent maternal care and normal social interaction during the food deprivation period, may be normal in tests for emotionality, exploratory, motor and learning behaviour, fertility and maternal and mating behaviour in adulthood. Possibly, the deficits and deviations which other experimenters have found in neonatally food-deprived animals have resulted from confounding variables such as poor or restricted maternal care, the absence of normal social interaction, or differences in the handling in infancy.

The absence of behavioural deficits in the present experiment is not attributable to an insufficient degree of food deprivation. In other investigations cited in this paper the percentage of control body weight attained by food-deprived animals ranged from 20 - 65% at weaning and 65 - 85% in adulthood. In the foster mother food deprivation experiment the experimental animals were 50% of control body weight at weaning and about 80% at the age of 115 days. Thus, the animals tested in this experiment were as severely stunted as those in other studies, and the major difference between this experiment and the others was the provision of a constant number of littermates and full-time maternal care to food-deprived animals.

The provision of a non-lactating foster mother during the food deprivation period was apparently adequate to prevent the development of learning deficits. This corroborates the findings of Nováková (1966, 1970) who showed that the presence of a nipple-cauterized mother during the second half of the suckling period (days 16 through 30) prevented the occurrence of learning deficits in rats weaned at 16 days of age. Whether the care provided by the foster mothers in the present study was in fact the same as the care provided by real mothers was not systematically recorded. However, other experimenters using the same procedure for inducing fostering as used in the present experiment, have reported that they could not distinguish between foster and real mothers (mice) in the quality or amount of care they offered (Beniest-Noirot, 1958; Gandelman, Paschke, Zarrow and Denenberg, 1970). Although in the present study maternal behaviour was not studied in detail, it

was noted that during the twice-daily switching period the foster mothers, as well as the real mothers, were usually crouched over the nest, and that pups were suckling both types of mothers.

Clearly, early social deprivation can also affect learning (e.g. Fuller, 1967) and emotionality (Seitz, 1954) in adulthood. Thus the effectiveness of normal social (including maternal) interactions in their prevention of disorders of learning and emotionality in food-deprived animals is not completely unexpected. Why the provision of littermates and maternal care should have prevented deficits in motor ability in the present study is harder to understand. Others have reported motor deficits in undernourished rats (low protein diet to the lactating mother) when tested in infancy (Altman, Sudarshan, Das, McCormick and Barnes, 1971). However, since they tested motor ability with learning tasks, the true deficits may have been in learning and not in coordination. This is supported by the fact that the motor deficits tended to disappear rather soon after nutritional rehabilitation (Altman and McCrady, 1972). Another explanation for the absence of motor deficits in the present study may be that the elevated runway, as well as climbing a horizontal string or ladder (Lynch and Smart, pers. comm. 1972), is not appropriate for testing motor coordination, because the undernourished animals may have the benefit of their smaller body size.

The occurrence of striking differences in activity levels between food-deprived and control animals corroborates findings reported by others (Franková and Barnes, 1968a; Guthrie, 1968; Barnett, Smart and Widdowson, 1971). The cause of the higher activity level in food-deprived animals is not known. It may be due to the curtailment of cerebellar cell development produced by neonatal food deprivation (e.g. Fish and Winick, 1968; Altman and McCrady, 1972); however, heightened activity has been found in animals deprived of food beginning on Day 28 (Barnett, Smart and Widdowson, 1971), by which time brain differentiation is almost complete and food deprivation causes no structural damage. Heightened food drive seems to be ruled out as an explanation, since the food arm in the plus maze was not visited more often than other arms. A permanent alteration in thyroid function by neonatal food deprivation may be yet another explanation, since it has been shown that neonatal administration of triiodothyronine resulted in permanent retardation of somatic growth and hyperactivity (also measured in the plus maze) at 90 days of age (Pelt, 1972). However, the serum levels of two

main thyroid hormones were not significantly different between food-deprived and control animals (see Table 46). Pelt also failed to find

| Experiment | Age | Sex | Treatment | N | Triiodothyronine ng/100 ml serum | Thyroxine µg/100 ml serum |
|---|---|---|---|---|---|---|
| Foster mother food-deprivation | 580 | ♂ | Food-deprived | 14 | 59 | 3.25 |
| | | | Control | 10 | 39.5 | 3.15 |
| | | ♀ | Food-deprived | 9 | 40 | 3.2 |
| | | | Control | 10 | 41 | 3.35 |
| Incubator food-deprivation | 260 | ♂ | Food-deprived | 12 | 30.5 | 3.0 |
| | | | Control | 6 | 22 | 3.5 |
| | | | All males | 18 | 29.5[a] | 3.1[b] |
| | | ♀ | Food-deprived | 10 | 49 | 3.6 |
| | | | Control | 6 | 49 | 3.65 |
| | | | All females | 16 | 49 | 3.59 |
| Big litter food-deprivation | 250 | ♂ | Food-deprived | 10 | 38 | 3.5 |
| | | | Control | 6 | 40 | 3.7 |
| | | | All males | 16 | 39 | 3.51[c] |

[a] significantly different from all females ($x^2 = 5.78$, $p < .02$)

[b] significantly different from all females ($x^2 = 6.06$, $p < .02$)

[c] significantly different from all incubator food-deprived males ($U = 73.5$, $p < .05$)

*Table 46. Median levels of thyroid hormones in serum of neonatally food-deprived animals.*

differences in thyroid activity in adult rats, as measured by serum thyroxine levels.

The greater activity in the plus maze and the shorter running times in learning tests may reflect the increase in excitability that has been reported to be characteristic of underfed animals (Ruch, 1932; Lát, 1967; Barnes, Neely, Kwong, Labadan and Fraňková, 1968).

The *incubator* food deprivation regimen caused stunting in physical growth and development which was of the same degree as that noted in the foster mother food deprivation experiment. No effects were found upon emotionality, exploratory behaviour and food-motivated maze

learning, but in visual discrimination learning the food-deprived male and female rats showed a deficit.

Although the pups were maternally deprived, their frequent handling may have prevented the development of abnormalities in emotionality and exploratory behaviour. This corroborates the findings of others (Franková, 1968, 1969; Levitsky and Barnes, 1972). They found that handling ("stimulating") of the pups during the suckling period can prevent behavioural effects of undernutrition imposed during this period. The regular handling (rather than the maternal care) may also be the cause of the lack of differences obtained in open field behaviours between experimental and control rats of the foster mother food deprivation experiment. Russel (1970) showed that maternal deprivation for 1 or 10 hours per day on Days 3 to 9 had no effect on ambulation scores in the open field at 70 days of age, although defaecation scores were significantly lower. However, since the control litters were not disturbed, the handling of the experimental pups may have prevented the occurrence of alterations in exploratory behaviour and the decrease in emotionality in maternally deprived rats.

A possible way to differentiate between effects of maternal care and handling would be to repeat the foster mother food deprivation experiment with the following modification: twice-daily switching of the mothers instead of the pups. This would be a food deprivation regimen with (a) constant maternal care, (b) constant litter size, and (c) no handling.

The absence of differences between incubator food-deprived and control animals in Hebb-Williams maze learning may be the result of an augmented food drive in the experimental animals. The fact that in the plus maze the experimental males and females visited the food and water arm more often than the two other arms could indicate an increase in the motivation towards food. A heightened food drive could thus compensate for a decrease in learning ability imposed by neonatal food deprivation. Thoman and Arnold (1968) have also failed to obtain significant differences in Hebb-Williams maze performance between incubator hand-reared and mother-reared littermates, when tested at the age of 70 - 80 days. Baird, Widdowson and Cowley (1971) on the other hand reported that malnourished animals performed less well in the Hebb-Williams maze than well-nourished rats. However, they tested the animals for the first time at the end of the malnutrition period, when it is very

likely that the experimental animals are still suffering from the food deprivation. Therefore, they have not provided evidence whether malnutrition early in life, followed by nutritional rehabilitation, affects learning ability in adulthood.

The occurrence of significant deficits in visual discrimination learning in incubator food-deprived male and female rats is not easy to explain. Food deprivation with permanent maternal care did not bring about these deficits in the experimental animals. Increased food drive in the experimental animals is ruled out as an explanation since during this test the animals were not reinforced by food, but the excape from an electric shock was the reinforcement. Therefore, since both groups were handled equally, it must be concluded that the impaired shock-motivated Thompson box performance was caused by maternal deprivation. Other experimenters, using a comparable incubator food deprivation regimen, failed to show deficits in adult visual discrimination tests (Rajalakshmi, Ali and Ramakrishnan, 1967; Howard and Granoff, 1968). However, in both experiments cited there was a conspicuous difference in handling: the control litters were seldom touched while the experimental young were handled frequently.

The plus maze activity scores show sex differences in response to incubator food deprivation. Males were significantly more active than their controls (which was to be expected in view of the results of the foster mother food deprivation experiment), while ovariectomized females were somewhat less active than their ovariectomized controls. The finding that the control females did not significantly differ in activity from either group of males accords with similar findings (after ovariectomy) in the foster mother food deprivation study. Why food-deprived females were significantly less active than food-deprived males is not known. It is not caused by an increase in thyroid activity. Rather, males had significantly lower levels of triiodothyronine and thyroxine than females (a finding which in itself can not be explained; there were no differences in the concentrations of thyroid hormone binding proteins in the blood between the various groups).

The animals of the incubator food deprivation study were about twice the age of the foster mother food deprivation animals at the time they were tested in the plus maze. Also the foster mother females were placed again in the plus maze about 2 weeks post-ovariectomy, whereas the incubator females, ovariectomized at the same age, were placed in

the plus maze some 100 days following the operation. These differences should be taken into account when comparing the plus maze results of the two experiments.

The *big litter* food deprivation experiment deserves some critical observations. In this experiment all 6 remaining animals of only one mixed litter of 8 served as a control group. It would have been better to have had several litters of 8 pups, and to have employed a number of animals chosen randomly from these litters as a control group.

Because of these considerations the findings must be interpreted with some caution. Nevertheless, it seems legitimate to discuss the findings since the somatic growth measures of this control group of 6, as well as the behavioural measures, are very similar to those obtained for the control males in the incubator food deprivation experiment.

One of the most striking differences between the results of the big litter food deprivation experiment and those of the other two experiments is the fact that big litter reared animals are less stunted in their somatic growth. The great variability in body measures at all ages confirms data reported by Park and Nowosielski-Slepowron (1971). These workers found that adequate feeding (litter size 6) results in rats being of uniform size, while inadequate feeding (litter size 18) gives rise to marked individual differences at the time of weaning. Seitz (1954) reported that differences in body weight between big litter (12) reared and "normal" litter (6) reared animals tended to disappear, in females already at 3 months of age, in males at one year of age. Other experimenters have also reported similar findings (D'Amato, 1960; Altman, Das, Sudarshan and Anderson, 1971).

In adulthood the big litter reared animals in the present study did not show abnormalities in emotionality, but they showed increased exploratory behaviour, improved food-motivated maze learning and increased plus maze activity. The significant rise in the ambulation score in the open field for the big litter reared animals contrasts with results obtained in other investigations (Seitz, 1954; Lát, Widdowson and McCance, 1960; Guthrie, 1968; Fraňková, 1970a). Although it might be thought that the discrepancy could be due to abnormally little exploratory behaviour in the control animals, there is in fact no cause for the latter supposition. These controls did not differ significantly from the controls of the incubator food deprivation study, while comparison with

the incubator females showed the normal sex difference: males ambulating less than females. If growing up in a large litter provides a greater amount of (stressful ?) stimulation to the animals, it may act the same way "early handling" does, alternatively, the big litter situation could act as an "enriched environment". There exists an extensive literature reporting that early handling and rearing in an enriched environment increases the open field activity in adulthood (e.g. Denenberg and Zarrow, 1970; Rosenzweig, 1971). This could explain the increased open field activity in the big litter food-deprived animals in the present study, but not the opposite findings referred to above.

The improved food-motivated maze learning in big litter reared animals observed in this study is of interest. In other studies on large litter reared animals, in which different learning tests were employed, learning ability has been found unaltered (avoidance learning: Guthrie, 1968; Franková, 1970; brightness discrimination: D'Amato, 1960; and T-maze learning: Seitz, 1954). Comparisons of error scores between control males of the big litter and of the incubator food deprivation experiments, revealed that big litter control animals did not make significantly more errors ($U = 9.5$, n.s.). Also the food-deprived animals of both experiments did not differ significantly in error scores ($U = 22.5$, n.s.). An increased food motivation (as judged by the preference for the food and water arm in the plus maze) and the enriched environment of the large litter could explain the improved maze learning ability in the big litter reared animals in the present study. This accords with the finding that "enriched animals" performed better in the Hebb-Williams maze than "impoverished animals" (Rosenzweig, 1971).

In the plus maze the big litter food-deprived males were significantly more active than controls. This corroborates the findings of Guthrie (1968), who used a Wahmann activity wheel for the measurement of activity.

## CONCLUSIONS OF PART B

Neonatal food deprivation *per se* does not affect learning or emotionality in the adult rat, but causes a permanent increase in locomotor activity. Traditionally used food deprivation regimens all confound

food deprivation with changes in social environment, including access to and quality or amount of maternal care.

The sensitivity of complex behaviour to deficits in the early social environment and maternal care dictates that the experimental techniques for producing food deprivation be carefully examined for their effects on the social environment, including maternal care, before conclusions be made about the effects of food deprivation on behaviour.

# SUMMARY

It is well known that in the rat, as in most other mammalian species, changes in endocrine, nutritional, and social environment during specific "organizing or critical periods" in pre- and/or postnatal life may permanently affect growth and development. In the first part of the present study an attempt has been made to establish the role of the gonads and of androgens during late prenatal and early postnatal life in the control of subsequent somatic growth and development (part A. Growth and development of male and female rats following various prenatal, neonatal and late postnatal endocrine treatments). In the second part of the present study the effects have been studied of food deprivation during early postnatal life with or without maternal and/or social deprivation on subsequent growth and development, and on behaviour in adulthood (part B. Growth and development of male and female rats following various regimens of neonatal food deprivation).

It is demonstrated in part A that male as well as female rats born with a small body size tend to become small adults, whereas animals big at birth grow up to become big in adulthood. This finding demonstrates the necessity for matching animals for body weight at birth, especially in studies into the effects of prenatal treatments on postnatal growth and development. The adult sex difference in body size, males larger than females, is due to a persistent difference in the rate of growth from the time of puberty onward. Gonadectomy within 24 hours of birth abolishes sex differences in body growth, whereas gonadectomy on the 21st day of life causes a diminution of the sex differences. There are no differences in growth pattern between females ovariectomized at birth and females ovariectomized at 21 days of age. In male rats this is different: males castrated at birth become lighter and smaller than males castrated on Day 21. This demonstrates the significant role of the neonatal testes on subsequent growth, whereas the prepuberal ovaries do not seem to play an important role. This conclusion is wholly confirmed by the results of the next two experiments.

Prenatal exposure to androgen (2 mg TP/day to the pregnant doe from days 16 - 20 of pregnancy) results in masculinization of the genitalia in the female offspring. It also causes male and female rats to be

born with subnormal body weights, probably because of nutritional deprivation of the foetus through damage to the placenta. The combined procedures of ovariectomy at birth, and prolonged neonatal androgen treatment (.5 mg TP/2 days from Days 2 - 20) result in a male type growth pattern: similar to that of males castrated at 21 days of age.

A single neonatal androgen injection (.5 mg TP on Day 3) causes female rats to become heavier and larger than oil injected controls. In females the combined procedures of ovariectomy at birth and a single neonatal androgen injection result, as in the previous experiment, in a growth pattern similar to that of male rats castrated at 21 days of age.

In part B it is demonstrated that undernutrition early in life (with a lactating mother for only 12 hours per day, from Days 2 - 25, or growing up in litters of 18 pups) causes permanent retardation of body growth in male and female rats. Females are less affected by food deprivation than male rats. Rearing in large litters (only males are studied) has a rather mild retarding effect on somatic growth and development and does not seem to be a valid food deprivation regimen in studies on the effects of undernutrition early in life.

Neonatal food deprivation combined with constant litter size and permanent maternal care causes male and female rats to display increased locomotor activity in adulthood (as measured in a residential plus maze). There are no lasting effects of neonatal food deprivation on emotionality and exploratory behaviour (tested in an open field), on learning ability (tested in the food-motivated Hebb-Williams maze and in the shock-motivated Thompson box), on motor coordination (tested with an elevated runway), on female fertility and maternal behaviour, and on sex behaviour in male rats.

Neonatal food deprivation combined with constant litter size and restricted maternal care causes increased adult locomotor activity (as measured in the plus maze) and impaired learning ability in a visual discrimination test (shock-motivated Thompson box). There are no lasting effects of this food deprivation regimen on emotionality and exploratory behaviour (open field), and learning ability in the Hebb-Williams maze.

Neonatal food deprivation combined with increased litter size and permanent maternal care causes increased locomotor activity (plus maze),

increased exploratory behaviour (open field), and improved food-motivated learning (Hebb-Williams maze).

It is concluded: (A) At birth both male and female rat brains are not yet "organized" as to the neural mechanisms regulating the sexual dimorphism in the postnatal growth pattern. After birth androgens produced by the prepuberal testes are responsible for the organization of the male growth pattern. The prepuberal ovaries have no organizing effect. After puberty testicular secretions stimulate, whereas ovarian secretions inhibit body growth. (B) Neonatal food deprivation *per se* causes permanent retardation of body growth in male and female rats, but does not affect the sex dimorphism in patterns of growth. Moreover, it has no lasting effect on learning ability or emotionality, but causes a permanent increase in locomotor activity. It is concluded that previously reported effects of neonatal undernutrition on behaviour in adult rats were primarily caused by alterations in the animals' early social environment such as maternal deprivation or membership in a large litter.

# SAMENVATTING

Het is een bekend feit dat veranderingen in hormonale, voedings en sociale omstandigheden gedurende bijzondere "organisatie of kritieke perioden" voor en/of na de geboorte, de groei en ontwikkeling van de rat, zowel als van de meeste andere zoogdieren, blijvend kunnen beinvloeden. In het eerste gedeelte van dit proefschrift is een poging gedaan na te gaan welke rol de geslachtsklieren en mannelijk geslachtshormoon spelen vlak voor en kort na de geboorte, in de lichamelijke groei en ontwikkeling (Deel A. "Groei en ontwikkeling van mannelijke en vrouwelijke ratten na verscheidene prenatale, neonatale en laat postnatale endocriene behandelingen"). In het tweede deel van dit proefschrift zijn de gevolgen bestudeerd van ondervoeding gedurende de eerste 25 levensdagen, met of zonder beperking van moederlijke zorg en/of sociale wisselwerking voor lichamelijke groei en ontwikkeling en voor gedrag in volwassenheid (Deel B. "Groei en ontwikkeling van mannelijke en vrouwelijke ratten na verscheidene methoden van neonatale voedsel-onthouding").

In deel A is aangetoond dat mannelijke zowel als vrouwelijke ratten die klein zijn bij de geboorte de neiging hebben klein te blijven als volwassen dieren, terwijl dieren die groot zijn bij de geboorte ook groot zijn in volwassenheid. Dit toont aan dat het noodzakelijk is dieren te groeperen volgens geboortegewicht, in het bijzonder in onderzoekingen die nagaan wat de gevolgen zijn van vóór de geboorte toegepaste behandelingen op de lichamelijke groei en ontwikkeling ná de geboorte. Het geslachtsverschil in lichaamsgrootte op volwassen leeftijd, mannetjes groter dan vrouwtjes, wordt veroorzaakt door een constant verschil in groeisnelheid vanaf de puberteit. Het wegnemen van de geslachtsklieren (gonadectomie) binnen 24 uur na de geboorte doet de geslachtsverschillen in lichaamsgroei verdwijnen, terwijl een dergelijke operatie op de 21ste levensdag een verkleining van de geslachtsverschillen te zien geeft. Vrouwelijke ratten wier eierstokken bij de geboorte zijn weggenomen (ovariectomie) en wijfjes waarbij dit op dag 21 is gebeurd, vertonen eenzelfde groeipatroon. Voor mannelijke ratten is dit anders: mannetjes die bij de geboorte gecastreerd zijn blijven hun hele leven lichter en kleiner dan mannetjes die op dag 21 zijn ge-

castreerd. Dit laat duidelijk zien dat gedurende de eerste weken na de geboorte de teelballen belangrijk zijn voor de lichaamsgroei na de puberteit, terwijl de eierstokken gedurende de eerste weken na de geboorte daarvoor van weinig belang lijken te zijn. Deze gevolgtrekking wordt nog eens bevestigd door de volgende twee onderzoekingen.

Het toedienen van mannelijk geslachtshormoon (androgeen) vóór de geboorte (2 mg testosteron propionaat (TP)/dag aan de drachtige rat van dag 16 tot en met dag 20 van de dracht) veroorzaakt een duidelijke vermannelijking van de geslachtsorganen bij de vrouwelijke jonge ratjes. Het heeft tevens tot gevolg dat de jonge ratjes bij de geboorte een veel lager gewicht hebben, waarschijnlijk veroorzaakt door slechte voeding voor de geboorte doordat de moederkoek niet goed functioneerde. De gecombineerde behandeling van ovariectomie bij de geboorte en langdurig toedienen van androgeen in de weken na de geboorte (0.5 mg TP om de dag, van dag 2 tot en met dag 20) veroorzaakt een mannelijk groeipatroon: gelijk aan dat van mannetjes die gecastreerd zijn op de leeftijd van 21 dagen.

Eén enkele androgeen injectie vlak na de geboorte (0.5 mg TP op dag 3) zorgt ervoor dat vrouwelijke ratten zwaarder en groter worden dan de met olie ingespoten controle-dieren. Ook hier zien we dat de combinatie van ovariectomie bij de geboorte en androgeen toediening vlak na de geboorte een groeipatroon veroorzaakt gelijk aan dat van op dag 21 gecastreerde mannelijke ratten.

In deel B is aangetoond dat ondervoeding gedurende de eerste weken na de geboorte (de jongen worden tijdens de eerste 25 dagen van hun leven slechts gedurende 12 uur per etmaal bij de melkgevende moeder geplaatst, of ze groeien op in nesten van 18 jongen) een blijvend groeibelemmerend effect heeft. Vrouwtjes ratten worden minder in hun groei geremd dan mannetjes. Het opgroeien in een groot nest (alleen mannetjes zijn bestudeerd) belemmert de lichamelijke groei en ontwikkeling slechts in geringe mate. Daarom lijkt het ondervoeden door middel van het samenstellen van grote nesten geen goede methode om de gevolgen van ondervoeding vroeg in de jeugd op groei, ontwikkeling en gedrag in volwassenheid na te gaan.

Ondervoeding in de jeugd, gecombineerd met constante nestgrootte en voortdurende moederlijke zorg (een niet melkgevende pleegmoeder gedurende de 12 uur voedsel-onthouding), veroorzaakt een toegenomen loop-acti-

viteit in volwassen mannelijke en vrouwelijke ratten (gemeten in een kruisvormige kooi: "plus maze"). Er zijn geen blijvende gevolgen gevonden van ondervoeding in de jeugd in emotionaliteit en exploratiegedrag (onderzocht in een "open field"), in leervermogen (onderzocht in de "Hebb-Williams doolhof" met voedsel als beloning, en in de "Thompson box" waarbij ontsnappen aan een electrische schok de beloning is), in coördinatie van spierbewegingen (onderzocht met een verhoogde "evenwichtsbalk": "elevated runway"), in vrouwelijke vruchtbaarheid en moederlijk gedrag, en in paringsgedrag van mannelijke ratten.

Ondervoeding in de jeugd, gecombineerd met constante nestgrootte en beperkte moederlijke zorg (gedurende de 12 uur voedselonthouding in een broedstoof zonder moeder), veroorzaakt een toegenomen loop-activiteit in volwassen mannelijke ratten (gemeten in een "plus maze") en een verminderd leervermogen in een visuele-onderscheiding test ("Thompson box"). Er zijn geen blijvende gevolgen waargenomen in emotionaliteit en exploratie-gedrag, en in leervermogen in de "Hebb-Williams doolhof".

Ondervoeding in de jeugd, gecombineerd met toegenomen nestgrootte en voortdurende moederlijke zorg, veroorzaakt een toegenomen loop-activiteit in volwassen dieren ("plus maze"), een toegenomen exploratiegedrag ("open field") en een beter leervermogen in de "Hebb-Williams doolhof".

Uit deze onderzoekingen zijn gevolgtrekkingen gemaakt: (A) Bij de geboorte zijn de hersenen van mannetjes en wijfjes ratten nog niet "georganiseerd" voor wat betreft de mechanismen die de lichaamsgroei besturen. Na de geboorte zijn androgenen uit de onvolwassen testikels verantwoordelijk voor de "organisatie" van het mannelijke groeipatroon. De onvolwassen eierstokken hebben geen organiserende betekenis. Na de puberteit bevorderen hormonen uit de testikels de lichaamsgroei, terwijl hormonen uit de eierstokken de groei remmen. (B) Ondervoeding in de jeugd veroorzaakt een blijvende groeiremming in mannelijke en vrouwelijke ratten, maar doet het geslachtsverschil niet verdwijnen. Het heeft geen blijvende gevolgen voor leervermogen of emotionaliteit, doch het veroorzaakt wel een verhoogde loop-activiteit op volwassen leeftijd. De door andere onderzoekers gerapporteerde gevolgen van ondervoeding in de jeugd op gedrag en intelligentie op volwassen leeftijd, moeten dan ook beschouwd worden als teweeggebracht te zijn door andere oorzaken dan ondervoeding.

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# CURRICULUM VITAE

De schrijver werd 21 augustus 1940 te Dordrecht geboren. Hij behaalde het einddiploma H.B.S.-B aan het Chr. Lyceum aldaar in 1958. In hetzelfde jaar werd begonnen aan de studie in de biologie aan de Rijks Universiteit te Utrecht. In 1962 legde hij het candidaatsexamen K af, een jaar later gevolgd door het examen M.O.-biologie. Het doctoraalexamen biologie werd afgelegd in 1965.

De promovendus is als leraar biologie verbonden aan het Voorbereidend Hoger en Middelbaar Onderwijs sedert 1961, aanvankelijk aan het Chr. Lyceum te Harderwijk en sinds 1962 aan de Scholengemeenschap "Groen van Prinsterer" te Vlaardingen.

Gedurende 1966 en 1967 was hij part-time als wetenschappelijk medewerker werkzaam bij het Instituut voor Antropobiologie van de Rijks Universiteit te Utrecht (directeur: Prof. Dr. J. Huizinga). Sinds mei 1967 is hij als wetenschappelijk medewerker verbonden aan de afdeling Endocrinologie, Groei en Voortplanting van de Medische Faculteit Rotterdam (hoofd: Prof. Dr. J.J. van der Werff ten Bosch).

Van augustus 1969 tot augustus 1970 verbleef hij als visiting scientist in het Oregon Regional Primate Research Center, Beaverton, Oregon, U.S.A., waar gewerkt werd onder leiding van Dr. Robert W. Goy en Dr. Charles H. Phoenix.