DEVELOPMENT OF THE RAT AND EFFECTS OF PRENATAL GROWTH HORMONE TREATMENT

to
my
mother
father
and
brothers

NORMAL PRENATAL AND POSTNATAL DEVELOPMENT OF THE HOODED RAT AND THE EFFECTS OF PRENATAL TREATMENT WITH GROWTH HORMONE

by

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ABSTRACT

The prenatal and postnatal development of the rat has been investigated under normal conditions and following treatment of the mother with growth hormone during pregnancy.

Whereas most structural measures of normal body and brain development changed in a continuous fashion through the fetal and neonatal stages, the net change in brain DNA (cellularity) was biphasic; the first phase occurring in the fetal stage and the second over the second and third postnatal weeks. Daily treatment of the pregnant rat with growth hormone had no detectable effect on litter-size, placenta weight, or body and brain measures of the fetus in late gestation. However, the treatment did produce two definite effects: (i) prolongation of the gestation period; (ii) an increase in body-weight of the gravid rat which was maintained, in part, throughout lactation. Autopsy on postnatal day 40 showed that a definite growth response had occurred.

Naturally occurring differences in maternal body-weight were subsequently studied. Body-weight of the mother was not related to gestation period, litter-size, or birth-weight of the offspring, but was found to be inversely correlated with nest-time. Heavy mothers spent less time with their litters, and imparted a slight developmental precocity to them, compared with light mothers. In the open-field in adulthood, activity level was correlated positively, and defecation level negatively, with the amount of time the mother had spent with the litter in the first two weeks of life. A temperature-dependent model for maternal nursing behaviour was proposed.

Prenatal treatment with growth hormone resulted in changes in maternal behaviour in the direction predicted on the basis of body-weight. Offspring from growth hormone mothers showed no developmental precocity when age was dated from conception. Previously reported differences in adult brain structure and behaviour of growth hormone offspring were attributed to a postnatal influence of the growth hormone-treated mother.

PREFACE

The original aim of this work was to extend the investigation of the effects of prenatally-administered growth hormone on the development of the rat. However, the emphasis of the dissertation centres on the normal structural and functional development of the rat in the critical stages of early ontogeny. It will be seen that in the course of the work, new problems and interpretations have arisen, and these will be discussed at the appropriate times so that the chronological and conceptual development of the thesis is preserved.

Following some initial comments on the definition and description of developmental processes and events, the Introduction begins with an historical overview of the main theories of development, and is intended to provide some background to present-day thinking on the subject. The recent emergence of the psychobiological approach to development is then discussed, followed by a review of experimental work aimed at inhibiting or facilitating the early development of the nervous system of the rat. The Introduction concludes with a detailed review of studies concerned with the effects of prenatally-administered growth hormone on the development of the rat.

The four experiments reported in the first section bear on the structural development of the rat in the prenatal, perinatal, and early postnatal stages, and an assessment of the effects of prenatally-administered growth hormone on prenatal development is made. In the second section of the thesis, four experiments are presented in which structural and functional development in the early postnatal period is investigated, together with behaviour in adulthood. Again, the effects of prenatal treatment with growth hormone are assessed.

The argument will be presented that variations in the normal development of the rat, and those which occur following prenatal treatment with growth hormone, are the consequence of interactive relationships between mother and litter in the preweaning phase of development rather than of influences exerted prenatally.

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INTRODUCTION

A. Development

A useful way of conceptualising developmental processes and events is to compare them with physiological ones, particularly in terms of their outcome (Weiss, 1939); whereas physiological events are usually of a conservative, repetitive and impermanent nature, those of development are typically progressive and unidirectional, and frequently lead to a permanent change in the organism. A comparison on the basis of time or rate finds that developmental processes are often slow in contrast to physiological processes although there is little algorithmic basis for such a distinction.[1] Neither can a strict distinction be made on the basis of biological significance although in general physiological changes tend to hold an immediate functional significance for the organism whereas those of development tend to precede function in a more long-term fashion.

The progressiveness of developmental changes is not always immediately apparent. For example, cell-death would appear to be a regressive event, yet it is a common phenomenon in the early

[1] On a broader plane, a conceptually similar scheme has been usefully employed by Russell (1957) in distinguishing the experimentalist from the developmentalist approach to the analysis of behaviour.

development of the nervous system and may play a critical role in early histogenesis (Glucksmann, 1951). Its progressive property may therefore be manifested in the shaping and subsequent differentiation of nervous tissue (see Levi-Montalcini, 1964).

Developmental processes and events lead to an ever-increasing specificity (Hamburger, 1957); they are, in the main, outwardly progressive in appearance, and signify for the organism an increasing independence from environmental influences and a greater capability for self-regulation (Nagel, 1957).

A wide variety of phenomena have been grouped under the rubric development. Bonner (1963) has suggested that developmental processes be divided into two broad categories. The first, the constructive processes, are those involved in the progressive construction or building of the organism and may be further divided into growth, morphogenesis, and differentiation. The second are limiting processes which check, guide, and channel the former and may be intrinsic (e.g., hormones, chemical inducers) or extrinsic (e.g., food availability, mechanical factors, temperature). When development is seen primarily as a process of regulation (Holmes, 1948) it is these limiting processes which give a developing system its dynamic properties, and lend to it what has variously been called: élan vital, entelechy, formative drive, vital principle, etc. Historically, it was the recognition of these intrinsic factors, in particular the action of hormones, which

led to the demise of vitalism (see Thompson, 1917).

On the whole, more interest and effort has been directed towards the understanding of the relationship between these limiting processes and the fast, constructive processes of early ontogeny than to the later and slower changes in adulthood and the destructive processes of aging. However, as the eighteenth century physiologist von Haller noted, decrementum is as much a part of development as incrementum. (cited in Thompson, 1917). The present distribution of effort is clearly due to a higher payoff at the level of early ontogeny and logically these studies should come first. It may be expected that as the picture of early development becomes more complete a greater amount of attention will be directed towards the slower developmental features of adulthood and senescence.

B. Theories of Development

Over the last twenty years, an increasing amount of attention has been directed towards the comparative psychobiology of development, and it is interesting and useful to distinguish this recent and highly experimental phase from the more protracted study of development in general. The account which follows is not exhaustive and deals only with the two basic, and opposed, notions of development which have recurred in various forms from the time of the Ancient Greeks to the present day. No attempt will be made either to detail the historical contributions from philosophers and others which have substantively influenced the evolution of ideas on the subject.

Theories of human development can be traced back to the earliest beginnings of embryological theory. It was a member of the Hippocratic school who Needham (1963) credits with being the first embryologist. This unknown writer made two basic contributions: first, he espoused a mechanistic and causal approach to the study of development; second, he delineated the preformationist approach which held that everything was originally and simultaneously present in the embryo and that development was simply an increase in size of these preformed parts.

In Book II of the final part of Aristotle's zoological works, written sometime in the fourth century B. C., preformationalism is contrasted with an alternative theory of development, epigenesis, which holds that new structures are formed during the course of development. Of the

two theories, Aristotle clearly favoured epigenesis:

> 'How, then, are the other parts formed? Either they are all formed simultaneously - heart, lung, liver, eye, and the rest of them - or successively, as we read in the poems of Orpheus, where he says that the process by which an animal is formed resembles the plaiting of a net. As for simultaneous formation of the parts our senses tell us plainly that this does not happen: some of the parts are clearly to be seen present in the embryo while others are not."
>
> --De Generatione Animalium, 734a

> "And it is possible that A should move B, and B move C, and that the process should be like that of those 'miraculous' automatic puppets: the parts of these automatons, even while at rest, have in them somehow a potentiality, and when some external energy sets the first part in movement, then immediately the adjacent part comes to be in actuality."
>
> --Ibid., 734b

All other past and present theories of development are derived essentially from these two basic concepts. For its time the epigenetic theory was surprisingly advanced but in the centuries which followed some sort of tacit preformationism seems to have gained sway and, with double irony, it was the discovery of the microscope which was to offer spurious proof to the Age of Enlightenment of man's preformed beginnings.

In the late seventeenth century, Malpighi (cited in Needham, 1963) made observations on the developing chick embryo and re-affirmed that development was an unfolding, and not an assembly of the constituent parts. Although his preformationism was moderate in that he recognised a disproportionate growth of the parts, his findings were

seized upon and elaborated into a more radical theory. The preformationists were divided into those who saw the preformed parts in the unfertilised egg (ovists) and those who saw them in the spermatozoa (animalculists). The theory received support from several claims that tiny men had been seen in the head of the human spermatozoon and that appropriate forms had also been discovered in the gametes of other species.

It was Wolff's observations (cited in Needham, 1963) on the development of the blood vessels of the blastoderm of the chick (1759), and on the development of the embryonic intestine (1768) which are held responsible for the demise of preformationism, although the theory survived for some further time. The physiologist von Haller, a convinced preformationist, believed that the intermediate stages of the unfolding process could indeed be discovered through "an unwearied and laborious patience," and in the following he takes a suggestion from Aristotle (loc. cit. 734a) and attempts to explain sexual conjugation which was clearly a prerequisite for development of the embryo:

> "It does not seem improbable, that the embryo, lying dormant during a long period, neither increases, nor is agitated, except by a very gentle motion of the humours, which we may suppose to oscillate from the heart into the neighbouring arteries, and from there back again into the heart. But it is also probable, that the stimulus of the male semen excites the heart of the fetus to greater contractions so that it insensibly evolves the complicated vessels of the rest of the body by the impulse of the fluid, and propagates vital motions through all the canals of the animal embryo."
>
> --von Haller, (1801) p. 434

Thus, preformationism was still alive in the nineteenth century, and even as late as 1888 His's espousal of epigenetic theory was, notes Thompson (1917), "met with harsh criticism and contempt."

Nagel (1957) has attributed the historical tenacity of preformationism to the fact that the concept of development contains the implicit idea of something latent finding progressive expression, and prior to the emergence of gene-theory it was more plausible to regard this covert, latent something as simply the reduced form of what was later to be manifest. The popularisation of preformationism gave rise to a scientific embodiment of the long-standing christian and philosophical view of man with his "naked reason" and his "innate ideas," and the theory also provided a structural basis for the transmission of original sin from generation to generation. In essence, preformationism was the spiritual ancestor of predeterminism and nativism.

The emergence of predeterminism in the late nineteenth century can be seen as a bridge between preformationism and radical nativism. Predeterminism held that physical and behavioural development were determined by preset biological factors and the rigidity of some "instinctive" behaviours appeared to lend support to the view. The nativistic doctrine, at least for some ethologists, similarly held that behaviour was the outcome of a passive translation of the genome (see later). Although, by the turn of the century the epigenetic view was predominant in embryology, recognition of this principle in a

broader context of development was limited to the few adherents of empiricism. With the advent of behaviourism, however, emphasis was thrown onto the role of the environment as a determinant of ontogeny (Watson, 1913; 1924). The polarisation between predeterminism and behaviourism was to set the stage for the nature-nurture controversy (for a discussion of the conceptual and semantic issues, see Lehrman, 1970).

The Russian approach to development (see Krushinskii, 1962) seems to have been one of diluted epigenesis, or an "interactionism" that effectively straddles the nature-nurture issue. Following the Pavlovian scheme, behaviour is considered to be made up of unconditioned and conditioned reflexes: the former congenital and the latter acquired. The simplest unit of behaviour is the "unitary reaction" which has a rigid and generally invariant expression in lower organisms. More complex behaviour in higher animals is seen to be composed of a complex of these "unitary reactions" and finds more variable final expression. The concepts of "innate" and "acquired" are retained but not separated from each other as all behaviour is seen to arise out of an interaction between the two. This view of interaction between the congenital and the acquired is with reference to units of behaviour and therefore differs fundamentally from the interactionist viewpoint that has been more commonly expressed by others. For example, in a discussion of the imprinting phenomenon, Moltz writes:

> "...there is still widespread agreement with the inference which Lorenz first drew concerning the manner in which the individual genotype is implicated in imprinting; the characteristic properties of imprinting are regarded as being functionally represented in an encapsulated set of genic determinants which are elaborated during embryogeny in a species-typic fashion.... In marked contrast to this nativistic view is an epigenetic conception that the imprinting pattern is organised during ontogeny through the progressive interaction between the developing organism and its sensory environment. The idea that there are genic determinants uniquely related to imprinting is rejected in favour of the view that imprinting, as well as any other species-typic response pattern, arises from the integrative influence on development of intra-organic processes and extrinsic stimulative conditions."
>
> --Moltz (1968) Pp 27-28

Thus, the fundamental epigenetic (interactionist) view holds that the species-typic response, and all other behaviour, arises out of the interaction and integration of intra-organic processes and external stimuli. In contrast, the nativist sees no such interaction; the genome is regarded as a "blueprint" for behaviour which requires no experiential stimuli for its expression. The Russian view is midway between the two recognising that a strict separation into the congenital and the acquired is not tenable as interaction occurs (but at a level at which behaviour is already present).

Rationally, it would appear that the genetic endowment of the individual organism imposes some definite limitation on its subsequent behavioural repertoire and that, from fertilisation onwards, the course of development and maturation will further delimit the behavioural outcome. In lower orders of animals it may be supposed that the

influence of extrinsic factors is minimal and there will result a more or less faithful representation of the genome in the relatively invariant species-typic behaviour. In higher animals, particularly those that are less precocious at birth, there will prevail a wider range of extrinsic stimuli than the prenatal environment could offer during the course of development; the influence of the environment would therefore be more critical and would result in less stereotyped and more variant behaviour.

This interactionist view appears to be the most widely held at the present time although the controversy, particularly with regard to human development, is not yet resolved. Perhaps it is the case (to stretch Haekel's dictum a little) that the present discord, concerning the determinants of ontogeny and its outcome, recapitulates the phylogeny of developmental theory.

C. Psychobiology of Development

The preceding, brief review provides some background to the recent emergence of comparative studies concerned with the psychobiological analysis of development. The strongest influences on this approach have without doubt come from recent advances in the fields of embryology and molecular and developmental biology over the last 70-80 years. In particular, the experimental approach in the psychology and neuropsychology of development has borrowed heavily on the techniques and conceptual approach of these sources (King, 1968). However, due perhaps to a greater diversity of interest in the area, acceptance of the epigenetic rationale has been incomplete and equivocal attitudes persist; tacit predeterminism and nativism remain evident in some contemporary approaches.

In general, the developmental approach aims at understanding the historical sequelae of sub-relationships involved in the formation of the final or overall relationship, and allows a more comprehensive interpretation of a phenomenon and the variables that most significantly affect it (Valenstein, 1968). One of the major inherent advantages in the psychobiological analysis of development is that the time dimension can provide potentially more information about a structure-function relationship than can an acute study of the same variables in the mature organism. Developmental investigations afford an opportunity to simultaneously examine the intra- and inter-systemic progressive

changes of structural and functional ontogeny, but this at some expense. The longitudinal approach inadvertently sacrifices some of the control over variables and conditions that may be achieved in the acute study and the developmentalist is forced, as Russell (1957) notes, "to deal with complex, relatively rough, imprecise, molar variables."

Some of this complexity and imprecision arises out of the fact that in early ontogeny many critical changes occur concurrently both within and between different systems, although some selectivity may be achieved where there is a marked systemogenic heterochronicity (see Anokhin, 1964). Also, it is often difficult to confirm causal relationships between variables and many observations are merely correlative. However, a second major advantage in the developmental approach is that the course of ontogeny may be modified by changing its direction, rate, and perhaps its outcome. If this may be achieved within a particular system, or better within a small part of that system, causality may be established although the gestaltist adage that "the whole is greater than the sum of the parts" is nowhere more appropriate than in the analysis of the interactive processes of early development:

> "...dynamic networks are, in general, refractory to analysis in isolated bits, pieces, and steps. By confining analytical studies to sufficiently small sectors of a network, one can identify self-contained 'causal chains', but as soon as one abandons that confinement of vision for a more comprehensive view, one recognises the fragmentary nature of these conclusions; for then one notes that those chains are intimately interlinked into a cohesive system of interactions by branches and anastomoses. Nonetheless, in tactical regards, the analytical procedure of singling out for study the simplest

fragments of such relational networks first, and only afterward paying attention to their cross-linkages, remains unexcelled. One merely must bear in mind that such a procedure requires a deliberate act of abstraction from 'context', neglect of which bars true understanding of any system, and of the nervous system in particular."

--Weiss (1970) p. 56

D. Early experience and critical periods

Considerable interest has been shown in the effects of early experience on the adult animal. This remains an analysis of the structure - function relationship but in a more generalised and qualitative sense than has just been described. The rationale, basically Freudian, holds that experiences in early life are most critical in determining the form and function of the adult. Ordinarily, development of the normal organism is buffered against mild fluctuations in the environmental milieu; a similar buffering occurs in the modification of the genotype as a mutant recessive allele must usually be present on both homologous chromosomes before it may be expressed in the phenotype (King, 1968).

The strongest point of the epigenetic argument is that if extrinsic factors determine the course of development they will optimally do so when the system still has sufficient plasticity to be moulded by such influences. As development is progressive, and as it proceeds most rapidly in early ontogeny, it is at this stage that the main effort has been made to characterise qualitatively and quantitatively the most relevant stimulus inputs and, accordingly, to demarcate "critical periods" in development.

The terms "critical moment" and "critical instant" were first employed by Stockard (1921) in an extensive treatise on the induction of monstrosities. They were used to characterise stages in early development usually during periods of high cell-multiplication, during

which external manipulation would have a maximal effect. Scott (1962) described three kinds of "critical period" for infantile stimulation, learning, and the formation of basic social relationships. He confirmed Stockard's (1921) view that the specificity of effects at particular times could be accounted for by the sensitivity of fast growing tissues to changes in extrinsic conditions. Fox (1966) described parallels in the pattern of postnatal neuro-ontogeny for several species which he adduced in support of the critical period hypothesis "in confirming that various aspects of neuro-ontogeny, in addition to the development of overt behaviour patterns, are organised and integrated in a definite interdependent sequence of events."

No attempt will be made here to review the literature relating to the "critical period hypothesis;" many of those concerned with development have stated their views on the subject and have attempted to define "critical periods"; polemics have developed around the issue (see Denenberg, 1964) and disagreement has arisen concerning the heuristic value of the concept (Scott, 1962; 1963; Schneirla and Rosenblatt, 1963).

For behavioural characteristics, critical period has been defined as "that period in development during which the probability of a behaviour pattern being emitted and the probability of it being reinforced by the environment are greatest" (King, 1968). During critical periods, the developing response is most frequently reinforced by the environment

and "canalisation" is said to occur. When canalisation is strong, the behaviour is rigid and highly specific and is referred to as a "taxis" or "instinct."

In the "early experience" paradigm, the influence of independent variables on a specific dependent variable is assessed during these "critical periods" or epigenetic crises. These studies have led to many new experimental and theoretical considerations pertinent to the developmental process although, as with many other studies in the general area of developmental psychobiology, they have been open to some general criticisms.

One of the most important, particularly with regard to the analysis of behavioural development, is that a descriptive rather than an analytic approach has often been taken (Schneirla, 1957). Developmental studies have typically adopted the principles but not the methodological rigour of experimental embryology and biology, and it is not uncommon to find accounts of abnormal development where the course of normal development has been insufficiently documented. Failures to replicate previous findings frequently occur, often because the parameters of normal development of a particular strain under specified conditions were not first delineated.

The second general criticism applies to the experimental control of the dependent and independent variables that are the object of study. The former are frequently assumed to be the only variables

undergoing manipulation, and the influence of covariant dependent variables, which may contribute to the outcome, either additively or through direct mediation, is sometimes overlooked. The independent variables may similarly lack specification, such that when a particular experimental manipulation is made, not only are particular independent variables changed but also other "independent" variables, which again may be the real mediators of the phenomenon under study. A particularly striking example of this involves the studies of undernutrition (see Plaut, 1970). There also exists a tendency to perform a number of diverse manipulations and group them all under the same label. The terms "handling," "gentling," "stressing," "trauma," "experience," "manipulation," "petting" etc. have all been used to describe experimenter-manipulations, which collectively have been referred to as "early handling" (see Schaefer, 1968), and it has been tacitly assumed that all such treatments have involved the same dependent variable. This may be true in a broad sense, although it seems likely that, given the qualitative and quantitative diversity of the treatments, other inter- and intra-systemic variables are also involved.

Notwithstanding these considerations, the developmental approach offers one of the most exciting challenges to contemporary psychobiological research. In the past the study of development has contributed to the dissolution of barriers between disciplines, and presently it is serving the same useful purpose within the area of comparative

psychobiology. There is probably more to the current interest in development than simply its heuristic value, and the impression is gained that the sustained interest comes from an appreciation of the dynamic vitalism of developing systems. The essays of Thompson (1917), Bonner (1963), and Weiss (1968) are examples of how the essence of growth and development, of both animate and inaminate things, has been appreciated in its broadest perspective.

E. Facilitative and Inhibitive Influences in Development

This section focuses on studies that have an immediate bearing on the present work, and will consider briefly some specific attempts that have been made to modify the developmental course of the nervous system of the rat. In general, the studies fall into two categories: those that have attempted to inhibit structural and functional development, and those that have attempted to facilitate it.

The terms "inhibitive" and "facilitative" carry the implicit assumption that departures from normality may be reckoned thus. Usually, the use of simultaneously-run control groups serves to establish some working criterion of what normal development is, and it is against these control values that the extent of facilitation or inhibition is estimated. However, the true significance of experimentally-induced changes in development is not clear unless the exact parameters of normality are known. In studies of development, it is often tacitly assumed that the earlier that something develops, or the more there exists of something at a particular stage in development, the "better" it is for the organism. Evidence will be reviewed later which challenges these two notions. Maximal may not necessarily be optimal for the organism, and, as is frequently the case in a highly inbred laboratory species, normal may not be optimal.

Inhibitive and facilitative influences on the development of the nervous system have been assessed at both the prenatal and postnatal

stages; sometimes, either deliberately or inadvertently, the two have been combined, e.g., if the diet of the mother is restricted during pregnancy prenatal development of the fetus may be inhibited, and if these offspring are subsequently reared by their natural mother, their development may be further restricted by lactational and other maternal deficits of a prenatal origin. Fostering paradigms may be used to partition out the respective prenatal and postnatal influences.

Generally, it appears that the effects of early manipulations hold permanent consequences for the organism and that, contrary to the traditional view that the fetus is in some way "spared" or protected from major environmental fluctuations (e.g., nutritional deprivation of the mother during pregnancy) it now appears that the developing brain is vulnerable to such influences (Dobbing, 1968). For example, the cell-population of the rat brain is virtually complete by about the end of the third postnatal week, and if hypoplasia is incurred during this "vulnerable period" of brain development it will probably be of a permanent nature; other deficits, established early in development, may also be irreversible. By the same token, it might be argued that the effects of enhanced development during this time are also permanent, although hypotrophy may result from disuse of excess elements and the system may subsequently tend towards "normal" limits.

Inhibitive Influences:-- No attempt will be made here to review the various treatments that have resulted in an inhibition of prenatal

development of the nervous system of the rat. The relevant work on even one of the treatment categories (see below) is vast and a detailed review would be outside the scope of the present work.

It is not difficult to interfere with the course of normal development so that it becomes sub-normal. The objective is simply one of disorganisation of the developmental process and basically requires no detailed knowledge of how the system works. Frequently, it is the case that disruptive influences have a generalised effect on the developing organism and this lack of specificity often diminishes the heuristic value of such studies. Attempts to inhibit the prenatal development of the nervous system of the rat may be categorised as follows:

(i) manipulations of maternal diet

(ii) administration of drugs to the mother

(iii) induction of hormonal changes in mother

(iv) general treatment of mother (e.g., stress)

(v) surgical intervention (e.g., ligation of uterine blood vessels)

(vi) intra-amniotic injections

(vii) ionising radiation

These treatments may be sub-divided on the basis of whether or not they directly involve the mother. Treatments (i) - (iv) all affect the fetus by altering the physiological state of the mother, whereas (v) and (vi) are principally local in their effect on the fetus, and (vii) may be either or a combination of both.

All treatments above have proved to be effective in inducing developmental abnormalities ranging from the overall reduction in growth of the brain that accompanies protein deprivation of the mother (see review by Altman, Das, and Sudarshan, 1970) to the effects of teratological agents (e.g., Barrow and Rowland, 1969). Although lack of specificity is one of the major drawbacks in such studies, some recent reports on protein deprivation during pregnancy have distinguished differential regional specificity (Schrader and Zeman, 1969; Winick, 1970). Further, the X-irradiation studies may prove particularly useful in correlating structure with function because of the developmental selectivity that may be achieved with the treatment (Hicks and D'Amato, 1961).

Facilitative Influences:--The treatments that have been used in attempts to "improve" the prenatal development of the nervous system are less numerous presumably because the goal is more difficult than disorganisation. The prospect has held considerable appeal however, not only because it lies at the other end of the experimental inhibitive-normal-facilitative continuum, but also because of the possibility of extrapolating the treatment effects to man. Zamenhof (1940) made the specific proposal that hormonal intervention during pregnancy might produce structural increments in the fetal brain which would result in an abnormally high level of adult brain function. Similar considerations were made at about that time (Dispensa and Hornbeck, 1941; Crile, 1941). More recently, Dalton (1968) and Money (1971) have considered

the possibility that intellectual gains in man may result from hormonally-induced changes in prenatal brain development. Block and Essman (1965) have suggested that hormonal mediation may be involved in the reported relationship between season of conception and human intelligence (Knobloch and Pasamanick, 1958) although the latter remains conjectural (see Joffe, 1969).

The experimental treatments that have been used so far in attempts to facilitate the development of the nervous system fall into two categories: (1) surgical reduction of litter-size, and (2) administration of hormones to the mother.

1. Reduction of litter-size:--Two surgical procedures have been employed: (a) unilateral ligation (UL) - one of the horns of the bicornate uterus is tied off at its cervical end prior to mating with the result that spermatozoa are restricted to one horn and only approximately half of the normal litter complement develops; (b) reduction during pregnancy - the gravid animal is laparotomised and a proportion of the litter is selectively aborted.

As part of an investigation into the ontogeny of adrenal cortical activity in the rat Milkovic and Milkovic (1959) performed UL prior to mating and confirmed that litter-size was reduced by about half at birth. UL-offspring were reported to be significantly heavier than control offspring on postnatal day 1, 50% heavier than controls by Day 3, and showed a two-day advance in the age of eye-opening and in

the age at which an adrenocortical response to epinephrine was elicited. The authors' conclusion that the UL procedure favoured prenatal development of the fetus and "even more their postnatal development" is perhaps open to criticism. While it is evident that some change in prenatal development occurred as a result of the treatment, it is not clear whether postnatal gains are due to the changes already present at birth or to the effect of being reared in a small litter, or to some combination of prenatal and postnatal influences.

Van Marthens and Zamenhof (1969) compared the development of fetuses from UL-mothers against those from sham-operated controls following delivery by caesarean section near term. Significant increases in body-weight, cerebrum weight and cerebrum DNA (cellularity) were reported in the UL-offspring. In a second group of similarly treated mothers who were allowed to deliver their litters normally, gestation period was reported to be normal, and the effects above, together with an increase in total brain protein, were confirmed. It was suggested that the observed increases were not a simple manifestation of the inverse relationship between litter-size and birth-weight that had been reported previously by others, and no evidence of such a correlation was found between natural litter-size and development. It was proposed instead that the effects were due to superior nourishment of the fetuses. However, the relationship between litter-size and birth-weight has recently been confirmed (Benson and

Morris, 1971; Smart, Adlard and Dobbing, 1972; Barr, Jensh, and Brent, 1973; Experiments VII and VIII of this work) and the possibility remains that the enhanced development of UL-fetuses and neonates reflects this effect.

One possible difficultly with the van Marthens and Zamenhof (1969) study is that few mothers were used and there is no indication that "litter-effect" (King (1969): when the between-litter variance exceeds the within-litter variance) was considered in the statistical treatment of the data. However, in a recent study (Croskerry and Smith, unpublished observations) in which litter-effects were controlled by pooling the data for three fetuses from each mother and considering the number of mothers as the sample size (see Abbey and Howard, 1973), significant body and brain weight increases were found near term in the caesarean-sectioned fetuses of UL-mothers.

Recently, Zamenhof (1971) has suggested that the developmental gains incurred by fetuses from UL-mothers may be due to increases in relative progesterone titers during pregnancy. In a normal pregnancy the ratio between the number of corpora lutea and the number of fetuses will be close to unity, whereas in the UL-case there would be roughly double the number of corpora lutea per fetus and hence a higher level of circulating progesterone relative to the number of fetuses. This hypothesis is interesting in view of some recent reports in man. Dalton (1968) reported developmental gains and higher academic ratings

at 9-10 years in the children of mothers who were treated for toxaemia of pregnancy with progesterone. In addition to an apparent dose-response effect, the greatest intellectual advances were observed in children from mothers who received the treatment before the 16th week of pregnancy. Interestingly enough, the first major period of cell-proliferation in the human fetal brain occurs from the 15th to 20th week of gestation and probably corresponds mainly to the production of neurons (Dobbing and Sands, 1970b).

Intellectual gains have also been reported among patients with adrenocortical syndrome in which there is an increased secretion of an androgen-like factor in fetal life (Money and Lewis, 1966), and in cases of progestin-induced hermaphroditism (Erhardt and Money, 1967). Reifenstein (1958) reported earlier maturation in the children of mothers treated for habitual abortion with 17-α-hydroxy-progesterone caproate. As Money (1971) has noted, however, it would be premature to regard these studies on humans as providing definitive evidence of a critical role for hormones in the prenatal determination of intelligence. The progesterone theory would first require that progesterone be a limiting factor in brain development and secondly that any early augmentation of structure be retained and later reflected in enhanced cerebral function. The theory has not as yet been tested and it may well prove untestable in humans.

Further work with animals may elucidate the role of progesterone

in development. In the pregnant rat, progesterone titers increase up to about the end of the second week and decline thereafter (Grota and Eik-Nes, 1967; Hashimoto, Henricks, Anderson, and Melampy, 1968). It is interesting to note that the first major phase of cell-production in the fetal brain begins at about the end of the second week of pregnancy (Croskerry, Smith, Shephard, and Freeman, 1973).

To summarise the unilateral ligation studies: it appears that by restricting the prenatal litter complement to approximately half, some developmental gain by term may be achieved although it is not yet known what is responsible for this augmentation of development. It is unlikely that these effects are due to an improvement in the level of fetal nutrition (see later discussion: Section 1), whereas there are some grounds for the belief that a change in hormonal balance is involved. It is important, however, to establish that the effects are not simply due to halving the size of the litter. Thus, it would be necessary to show that the development of fetuses from UL-mothers was augmented in comparison to that of fetuses from sham-operated mothers bearing naturally-small litters, i.e., of comparable size. Whatever is responsible for the effect, the UL technique affords an opportunity to examine the limiting processes of prenatal development.

The second method involves surgical reduction of litter-size during the course of pregnancy. Zamenhof and van Marthens (1971) mentioned unpublished experiments showing that if all but one of the embryos were

destroyed by cauterisation, the surviving fetus at term showed 19% more brain DNA than controls. Cauterisation was performed on Day 7 of pregnancy (personal communication). In a follow-up study using rabbits, reduction of implants to one on Day 9 of pregnancy (normal gestation = 32 days) resulted in significant increases in placenta weight, body-weight, cerebral hemisphere weight, and cerebral DNA and protein in the surviving fetus at prenatal day 30 (van Marthens, Grauel, and Zamenhof, 1972). It was reported that the extent of litter reduction was uncorrelated with the degree of enhancement of these parameters, e.g., the single survivor of a litter of four (75% reduction) showed a comparable cerebral hemisphere weight to that of a survivor from a litter of twelve (92% reduction).

The possibility that the two techniques, unilateral ligation, and restriction during pregnancy, might differ from each other only quantitatively led to a study of the effects of differential restriction of litter-size during pregnancy in the rat (Croskerry, Hall, Leon, and Smith, manuscript in preparation). Mothers were laparotomised on Day 10 of pregnancy and reduction in the range 0-90% was achieved by crushing implants with forceps. Following delivery by caesarean section on Day 21 it was found that placenta, body and brain parameters had all increased in a roughly exponential fashion with a decrease in litter-size, but only when the reduction was in excess of about 60% did the values of these parameters significantly exceed those of sham-operated

controls. Again, the mechanism underlying these effects is obscure. It may be that progesterone is involved, that alterations in the hemodynamic properties of the uterus occur or that, as a result of placental hypertrophy, fetal nutrition and metabolism is somehow improved. It would be interesting to delay the operation until after Day 10 as up to this time the procedure of crushing the embryo results in hypotrophy of the placenta. If the operation was delayed until Day 12 by which time the allantoic circulation is established, the placenta would survive and grow in a nearly-normal fashion to term (Huggett and Pritchard, 1945). Thus fetal growth could be assessed in the presence of a supernumerary complement of placentae and presumably too under the influence of an excess of chorionic hormones (see Petropoulos, 1973).

2. Administration of hormones to the mother:--Besides the suggested involvement of progesterone, the only other hormone which has been implicated in the prenatal development of the nervous system is growth hormone. Surprisingly, little research has been done on the possible effects of other hormones though a number of studies have been concerned with the experimental analysis of the finding in man that infants from diabetic mothers are frequently heavier than those from normal mothers (see Angervall, 1959; Hoet, 1969). Several studies in the rat in which a mild diabetic state has been induced either with alloxan (Solomon, 1959; Lazarow, Kim, and Wells, 1960) or streptozotocin (Pitkin, Plank, and Filer, 1971) have reported significant

increases in fetal weight near term or in neonatal weight following delivery. When a more severe state of diabetes was induced (Lichtenstein, Guest, and Warkany, 1951; Kim, Runge, Wells, and Lazarow, 1960; Powell, Caulfield, and Field, 1967) a reduction in fetal growth was found which has been attributed to reduced maternal blood sugar (see Hoet, 1969). Higher fetal mortality rates and prolongation of pregnancy are probably responsible for inconsistencies in the various reported effects of experimental diabetes (Kim, Runge, Wells, and Lazarow, 1960).

The prevailing explanation for facilitated fetal growth in experimental diabetes appears to be that the hyperglycaemic state of the mother produces fetal hyperglycaemia resulting in increased glucose utilisation by the fetal tissues and therefore increased growth of the fetus. In support of this view, Picon (1967) observed an increase in fetal growth following direct injections of insulin into the fetus in late pregnancy. In diabetes, the fetal pancreas may respond to hyperglycaemia by producing more insulin which has a lipotropic and anabolic effect (see Jost and Picon, 1970) and it is of interest that Angervall (1959) has reported a positive correlation between pancreatic islet volume and birth-weight.

Studies which have observed increased fetal growth following administration of cortisone to the gravid rat (Evans and Clingen, 1953; Angervall and Martinsson, 1969) may be related to the above in that it

has been suggested that cortisone produces hyperglycaemia which results in hyperinsulinism (Angervall and Martinsson, 1969).

In none of these studies in which increased fetal growth has been observed has there been an assessment of brain growth. If the increase in fetal weight is not due simply to an increase in fat or water content, and is a reflection of genuine growth as Picon (1967) has claimed, then a corresponding proportionate growth of the brain might also be expected.

As the foregoing indicates, the extent of investigations into the effects of hormones on prenatal development has been limited. A surprisingly extensive literature does exist, however, on the effects of growth hormone on fetal development and, in particular, on the development of the fetal brain. A detailed review of the literature on this subject is presented in the next and final part of this introduction.

F. Review of Studies on the Effects of Prenatal Treatment with Growth Hormone

Research into the effects of preparations from the anterior pituitary gland on prenatal development of the rat commenced almost fifty years ago. Over the last twenty-five years relatively pure preparations of growth hormone have been used but, to date, no clear concensus has emerged on the effects of the treatment (Cragg, 1972). Despite the uncertainty that exists in this area of research some of the more recent findings have been widely accepted in review articles and texts dealing with hormonal influences in development.

It will be seen in the present review that the area has been encumbered with considerable difficulties, some of which have been technical, e.g., contaminants in the various hormone preparations, procedural differences in administration, differing laboratory conditions, strain differences and so on. In addition, however, some of the methodological problems that were mentioned earlier have also been present.

Quite apart from the obvious experimental potential of this type of research, it appears that some other zeitgeist, perhaps the possibility of radically modifying the structural and functional organisation of the brain, has invited the interest of several disciplinary groups over the years. Others, too, have been inspired by the possibilities:

> "By introducing a certain hormone into the bloodstream of the mother he could affect the growth of the brain of the unborn young. The hormone apparently had a double effect. It increased the actual bulk of the cerebral cortices and also it made the nerve

> fibres themselves much finer than they normally are, so that a far greater number of them, and a far greater number of connexions between them occurred in any given volume of brain...
>
> A large population of rats and mice were sacrificed in an attempt to perfect Trelone's technique. At last he was able to produce a number of remarkable creatures. His big-headed rats, mice, guinea pigs, rabbits, though their health was generally bad, and their lives were nearly always cut short by disease of one kind or another, were certainly geniuses of their humble order. They were remarkably quick at finding their way through mazes and so on. In fact, they far excelled their species in all the common tests of animal intelligence, and had the mentality rather of dogs and apes than of rodents."
>
> --Olaf Stapledon (1944) Pp. 13-14

Following the classic demonstration of the growth promoting properties of anterior pituitary extract in rats by Evans and Long (1921), the first studies on the effects of the extract during pregnancy were performed by Teel (1926). Injections of the mother from the first day of pregnancy resulted in her gaining more weight than controls, and in the prolongation of pregnancy by 4-8 days with the mother giving birth to stillborn fetuses. Autopsy on Day 9 of pregnancy showed no implantation sites in several animals and it was concluded that the prolongation of pregnancy was due to delayed implantation. However, when injections were not commenced until after implantation had occurred, gestation was still found to be prolonged. Enlargement of the ovaries was believed to be involved in the failure to give birth normally at term. Greater fetal weight at term was found in the hormone-treated group. "Term" was defined as the time at which the mother's body weight became stationary or began to drop.

Hain (1932a) injected a similar preparation and reported a body-weight increase that was related to dose level. The residual weight-gain in mothers following parturition was found to be greater than that for controls. In a second paper (1932b) she reported prolongation of pregnancy, followed by stillbirths, and marked luteinisation of the ovaries. Using a gonadotropin-free preparation, no effect was found on the ovaries but again pregnancy was prolonged. Although the preparation was without effect on the ovaries of immature rats, it did affect the ovaries of mice and it was concluded that the preparation was still gonadotropic and was responsible for the prolongation of pregnancy. In a third study (1934) no effect of prolonged gestation was found with either the extract or "Phyone" (a growth hormone preparation from the Wilson Laboratories) in ovariectomised rats.

Sontag and Munson (1934) administered antuitrin-G (growth hormone, Parke, Davis and Co.) from the 8th to the 22nd day of pregnancy. The majority of their experimental mothers showed prolongation of pregnancy by 1 day or more past "normal term" but birth-weights of pups from these extended pregnancies were not significantly greater than those of experimental mothers who gave birth at normal term. The mean birth-weight of all pups from the hormone-treated mothers was significantly greater, and the incidence of still births three times higher, than controls. Offspring from hormone-treated mothers remained significantly heavier as far as postnatal Day 10. Although the authors inferred that

the hormone preparation had promoted fetal growth, the higher incidence of still births, and the extended gestation period coupled with imprecise dating of neonatal age, preclude any firm conclusion on this point.

In 1935, Watts administered preparations of GH (VanDyke and Wallen-Lawrence) and Phyone. All preparations produced significantly greater increases in maternal body-weight, but some prolonged pregnancy by 2-5 days resulting in stillborn young. Others had the same effect on maternal body-weight, but did not prolong gestation (21 and 22 days were considered normal) and produced significant increases in birth-weight of offspring.

To summarise the findings of the work in the 20's and 30's: the administration of extracts from the pituitary gland and crude preparations of growth hormone to the pregnant rat in most cases appears to prolong pregnancy and is associated with an increase in the number of stillbirths. When the hormone is administered early in pregnancy, some of the extension of the gestation period is attributable to delayed implantation. The extension of gestation that occurs when the hormone is not given until after implantation has occurred is due either to contaminants of the preparation or to growth hormone itself.

That the duration of pregnancy was not precisely measured in any of these studies calls into question the definition of "normal" term. Although the rate of fetal growth may be attenuated in late gestation, a difference in gestation period of less than 24 hr. would give rise to

highly significant differences in birth-weight, and would thus invalidate any firm conclusions concerning the effect of growth hormone on fetal growth. The finding of an increase in body weight in experimental mothers over controls appears to be consistent and reliable.

In 1940 there appeared a provocative paper which generated a new emphasis in this area of research. Entitled "On Present Possibilities of Increasing the Higher Functions of the Cortex Through Artificial Changes in its Architectonic" it contained the specific proposal that human intelligence could be increased by hormonal intervention in normal pregnancy. It was suggested that through the administration of growth hormone to pregnant women at a time before the number of neurons had become complete in the fetal brain (estimated to be about the 4th to 5th month (cf. Dobbing and Sands, 1970)) there would result a stimulation and prolongation of the proliferative phase resulting in a significant hyperplasia. This in turn would result in a greater number of connections and an increase in "...the degree of organisation of the human cortex and psychic life..." (Zamenhof, 1940, p. 25).

The author cited the results from the earlier experiments with rats (Teel, 1926; Hain, 1932, 1934; Sontag and Munson, 1934; Watts, 1935) as proof of the growth-promoting properties of growth hormone on the fetus, and suggested that the difficulties encountered (extended gestation, stillbirths) could be overcome with further purification of the hormone. It would then be possible to successfully produce the

superhumans described or "...to create a new species." (loc cit, p. 21).

The experiments which followed in the early 40's were clearly attempts at validating the above proposals in animals of 'lower' phylogenetic order. In the case of amphibians, substantial increases in brain weight and body weight had been associated with the pathological condition of hypertrophy of the pituitary (Hahn, 1912) and several experiments had indicated that gigantism could be produced in tadpoles and other larval amphibia through the administration of growth hormone or pituitary extract. In 1941 Zamenhof reported that the administration of Antuitrin G (a growth hormone preparation) into tadpoles produced significant increases (44-126%) in the numbers of neurons in the cerebral hemispheres and it was concluded that "...by means of the growth hormone it is possible to increase artificially and deliberately the number of brain neurons..." (p. 137).

In the following year it was reported that similar effects could be demonstrated in the rat. Following the daily administration of Phyone or Antuitrin G (preparations of growth hormone) from the 7th to the 18th to 20th day of gestation, significant increases were found in body-weight and in hemisphere weight, cortical area and thickness, cell-density and cell-population in the brains of caesarean-delivered offspring. In adulthood, significant increases were found in cell-density and in the total number of cells in the cerebral cortex. It was concluded that the proliferation of prospective cerebral neurons had been artificially

stimulated at the fetal stage through the administration of the growth hormone preparation to the mother (Zamenhof, 1942).

Several points deserve comment in this study. First, "normal term" was considered as Day 22 or Day 23, and the GH-treated offspring were obtained following caesarean section at "term" as the first experimental animals showed prolongation of pregnancy. The control offspring were presumably delivered normally. As was mentioned earlier, slight differences in the fetal ages of the two groups would give rise to spurious differences in body-weight and brain parameters between the two groups. The conclusions about the newly born fetuses are therefore in question.

Second, the offspring from growth hormone-treated mothers, upon which the estimates of cerebral cell-number and cell-density were made in adulthood, may not have been a representative sample. Although almost all the GH-offspring survived caesarean delivery, many died within the first few hours, and a majority of the survivors died within the first twelve days. The few that did survive into adulthood were the most viable and therefore atypical of the original population. In addition, preweaning mortalities in the GH-pup population would have resulted in reduced litter-sizes which, it is now known, would favour postnatal development (Heageness, Bindshadler, Chadwick, Conklin, Hulnick, and Oaks, 1961; McCance and Widdowson, 1962; Winick and Noble, 1966b; Dobbing and Sands, 1971). Conclusions based on these adult animals are therefore open to serious question.

Other survivors from this study were investigated in a maze-learning task in adulthood. Experimental subjects were reported to make fewer errors and require fewer trials to criterion than controls although the differences did not achieve statistical significance. It was concluded that the altered brain structure had no effect on maze performance but the possibility was raised that the task may have been overly simple and therefore unsuitable for the discrimination of differences in intelligence (Warden, Ross, and Zamenhof, 1942). Structuro-functional correlates were not investigated for the next 20 years.

Hultquist and Engfeldt (1949) gave Phyol (Alfred Benzon, Copenhagen) and Antuitrin G in increasing doses through pregnancy and found that both preparations prolonged pregnancy. Fetuses delivered by caesarean section were reported to be heavier than normally-delivered control fetuses but, again, there is the problem of precise dating of fetal age.

Frazer, Huggett, and Wohlzogen (1949) reported that the daily administration of 0.3 cc. of crude extract of the anterior pituitary (Young. 1941) from Day 10 to Day 20 had no effect on the body-weight of fetuses from mothers killed on Day 21. Significant increases in maternal body-weight were observed in mothers receiving the extract compared with control mothers. The authors concluded that either growth hormone did not cross the placenta, or that if it did, it failed to excite fetal growth.

At about this time relatively pure crystalline preparations of

growth hormone became available. Li, Evans, and Simpson (1945) were the first to achieve the isolation of growth hormone as a pure protein. An improved extraction procedure producing higher yields of the crystalline preparation, which was judged to contain very little contamination by other pituitary hormones, was later described (Wilhelmi, Fishman, and Russell, 1948).

Barns and Swyer (1952) injected a purified preparation of growth hormone (Armour Co.) into rats from Day 14 to 20 and reported a significant increase in fetal weight following caesarean section. However, litter size was reduced in the experimental group and a correction for this effect rendered the difference in fetal weight insignificant.

Engfeldt and Hultquist (1953) administered a purified crystalline preparation of growth hormone to groups of mothers of different body-weight and reported no differences in gestation period compared with controls. The hormone was found to produce significant increases in body-weight of only those fetuses from mothers of low body-weight over fetuses from the associated control group. Fetuses from experimental mothers in the middle or upper range of body weight were no heavier than their respective controls. It should be noted that fetuses from low body-weight control mothers were abnormally light in weight and the effect of GH in this weight range may have been to ameliorate this effect of low maternal body-weight. GH fetuses from low body-weight mothers were comparable in weight to those from control mothers in

the middle and upper range of body-weight.

Campbell, Innes, and Kosterlitz (1953) injected two mothers with 2.5 mg. purified growth hormone (Armour) daily between the 13th and 21st day and reported an increase of about 10% in fetal and placental weight over controls. However, from their data it appears that mean litter-size was about five less than that of controls and this may have accounted for the effect as a negative regression of litter-size on birth weight has been reported (King, 1915; Benson and Morris, 1971; Smart, Adlard, and Dobbing, 1972).

Cotes (1954) administered a crystalline preparation over the last twelve days of pregnancy at dose levels of 0.2, 0.5, 1.0, and 2.0 mg.daily. Mean pup weight at caesarean-section was found to be significantly greater than controls at the highest dose-level but again the possibility was raised that the effect might have been due to a reduction in litter-size.

In the same year it was reported that prenatal treatment with growth hormone caused fetal death in rabbits (Hoet and Brasseur, 1954), and resulted in prolonged gestation, fetal gigantism, and high rates of resorption and fetal mortality in rats (Nixon, 1954). Fetal gigantism and increased fetal mortality were also frequently reported in the pregnancies of pre-diabetic and diabetic women (for references see Jackson, 1954) and led Nixon (1954) to support the speculation of Young (1951) and others of a sustained hypersecretion of growth hormone in

pre-diabetic and diabetic women (see later discussion: Section 1). Chutkow, York, Plotz and Davis (1955) found no effects of growth hormone on fetal development in the rat.

Tuchmann-Duplessis and Mercier-Parot (1955) administered 10 I.U. of growth hormone (Choay Laboratories) daily to rats commencing on the 3rd, 4th, 5th, 6th, 7th, or 8th day of pregnancy and terminating on Day 16. All GH-treated mothers showed a greater than normal increase in body-weight during pregnancy, and in those allowed to deliver gestation was prolonged by 3-6 days and large stillborn fetuses were delivered. Fetuses from GH-treated mothers which were delivered by caesarean-section on Day 20 actually weighed less than controls of the same age. As the greatest reduction in fetal weight was observed when the GH-treatment was begun before Day 5, it appeared that growth hormone or some contaminant(s) of the preparation had delayed implantation. Other possibilities were considered that increased maternal protein anabolism may have reduced the supply of nutrients to the fetus, or that the GH-treatment may have induced some early metabolic disturbance in prenatal development. No effects on litter-size were reported. It was concluded from the results of these experiments, and from others involving maternal or fetal hypophysectomy, that hypophyseal growth hormone did not stimulate fetal growth and even appeared to retard it. However, as these authors had noted that the results of earlier work with the rat had implied a transplacental passage of the hormone (and

hence a direct action on fetal growth), it is somewhat surprising that the hormone treatment was stopped on Day 16 and not given during the rapid phase of fetal growth over the last five days of pregnancy.

In 1961, Clendinnen and Eayrs undertook an extensive study of the anatomical, physiological and behavioural effects on the offspring of mothers prenatally treated with growth hormone. 3.2 mg of the highly purified preparation (National Institutes of Health, U. S. A.) were administered daily from the 7th to 19th days of gestation. Mean birth-weight of offspring from GH mothers was reported to be significantly greater than that of controls. No data on gestation period or litter-size were reported. In the postnatal period, litters were culled to five and reared by their natural mothers. GH-offspring were reported to show slightly earlier appearance of the righting and placing reactions, and in adulthood made fewer errors in the Hebb-Williams closed field test of animal intelligence (Rabinowitz and Roswold, 1951). No significant change in the electroencephalogram was observed other than an abnormal response to photic stimulation. Quantitative histology of the cerebral cortex performed at 33 and 60 days revealed a 20% increase in the cell-gray coefficient in the experimental animals. This was attributed to hypertrophy of the cortical perikarya rather than hyperplasia as the relative number of perikarya was not increased. A hypertrophy of the dendritic field was found; the mean number of dendrites associated with each neurone was increased by 22%. Computation of the statistical

probability of interaction between cells (Uttley, 1955) revealed a marked increase (55%) in the experimental group over the controls.

In response to this failure to find evidence of neuronal hyperplasia, Zamenhof's group repeated the experiment using hormone derived from the same source, and combined biochemical and histological techniques to investigate postnatal changes in the brain. 3 mg. of the hormone was administered daily either subcutaneously or intraperitoneally from the 7th to the 20th day of gestation. Delivery was reported to be "normal and at term." Slight increases in body-weight in the experimental group were not significant but highly significant increases in cerebral hemisphere weight were found. Total DNA content, which provides an estimate of the total number of brain cells, was significantly increased. The effects on the brain were more pronounced with the intraperitoneal route than with the subcutaneous route. At 20 days of postnatal age cortical cell density was 63% higher in the subcutaneous group compared with controls and a 71% increase was found in the neuron-glia index (Zamenhof, Mosley, and Schuller, 1966).

Several aspects of this study deserve comment. First, there is again the difficulty in defining normal "term." Normal pregnancies may vary by as much as 50 hours in length (Kim, Runge, Wells, and Lazarow, 1960) and it would be helpful if the criteria for "normal term" were indicated for these particular experimental conditions (although the insignificant difference in birth-weights between the two groups in the

study does at least provide some indication that gestation periods were comparable). In view of the earlier reports of increased fetal resorption and mortality following growth hormone treatment, it would also be useful to know if similar effects were obtained here as this might conceivably influence the results. No data were presented on the incidence of still-births or litter-size.

With regard to the results reported on the offspring at 20 days two points should be made. First, there is no indication that litter-size was controlled in the postnatal period. Second, the histological data are based on only four control and three experimental animals, and no details are provided of the sampling procedure, i.e., whether the offspring were derived from several litters or from one litter. As King (1969) has observed, "litter effect," unless adequately controlled, can exert a considerable effect on the outcome and interpretation of developmental studies (see also Abbey and Howard, 1973). In addition, Jacobson (1970) has pointed out that the cell-density data obtained at 20 days may be open to question as no correction was made for brain shrinkage during fixation (see Abercrombie, 1946).

Attempts to establish a relationship between these reported alterations in structure and behaviour were made in three other studies. Block and Essman (1965) administered a much lower daily dose (100 μg) of the hormone (NIH). No effects on litter-size or gestation period were reported. At 56 days offspring were tested in a single-trial avoidance

task. Latency and extinction behaviours were significantly different between the two groups, although no differences had been demonstrated in an activity level test administered prior to the avoidance task. The results were interpreted to reflect adaptive superiority of the GH-offspring, as, following a single aversive experience, they more readily modified their behaviour making it more appropriate to the subsequent situation.

Ray and Hochhauser (1969) gave 3.2 mg. of the hormone (NIH) daily from the 3rd to the 19th day, and reported no differences in litter size or sex ratio between experimental and control groups. No differences in gestation period were reported. No litter-weight differences were found on Day 1 or at any time in the postnatal period. The offspring from GH-treated mothers showed significant gains in the age of appearance of the startle and righting responses compared with controls. On postnatal Day 21 offspring were assigned to either an "enriched" or "isolated" environment and in adulthood were tested in the open field, Lashley III maze, and shuttle-box avoidance. The open field results yielded a significant triple interaction: Days of testing x Sex x GH, but GH was not significant as a main effect. In the Lashley III maze, the GH treatment was found to be significant; GH subjects required fewer trials to criterion than did controls. Double interactions: sex x GH, and GH x environmental rearing condition, were also significant. In the shuttle-box avoidance task the GH effect figures significantly in two, three, and four way interactions with environment, sex, and days.

However, a failure to find behavioural differences resulting from the treatment has been reported (Gill, Reid, McClellan, and Porter, 1967). 3 mg.of the hormone was administered daily from the 7th to the 15th day of gestation. No differences in gestation period, litter-size, or birth-weight were reported. In adulthood GH-offspring were no different from controls on tasks involving either active or passive avoidance, or in performance in the Hebb-Williams maze. No differences in brain-weight or brain DNA content were found at sacrifice.

In other studies conducted with the purified preparation, Quinto, Bottiglioni, and Orlandi (1960) found an increase in birth-weight of offspring from rabbits treated with growth hormone. Angervall and Lundin (1962) hypophysectomised rats on Day 12 or 13 of pregnancy and administered thereafter twice-daily doses of approximately 0.25 mg.of porcine growth hormone (Ferring: "Somacton"). Fetuses were delivered by caesarean section on Day 22. No significant differences were found in birth-weight or birth-length between offspring from hypophysectomised mothers and those from hypophysectomised mothers who had received growth hormone. Litter-size was comparable in both groups.

Heggestad and Wells (1965) studied the "direct" effects of growth hormone on fetal development of the rat in late gestation. It was reported that whereas hypophyseoprivus (fetal hypophysectomy by decapitation) on Day 18 (452 hours from witnessed mating) resulted in a 20% reduction

in fetal weight and tibial volume by Day 21 (519 hours), the same operation followed by four subcutaneous injections of the fetus (one every 12 hrs) with 0.5 mg. growth hormone (Armour) resulted in a 12% increase in fetal weight by Day 21 although tibial volume remained slightly less than normal. A control group in which hypophyseoprivus was followed by four injections of albumen showed a 20% reduction in fetal weight on Day 21. It was concluded that the fetal hypophysis was responsible for 20% of fetal growth in late gestation, and that exogenous growth hormone could be used to replace the fetal hypophyseal hormones. As injections of the intact fetus with growth hormone produced no augmentation of fetal development, it was further concluded that fetal growth proceeded maximally under the influence of the fetal hypophysis.

To summarise the results of these studies conducted since the purified preparations of growth hormone became available: after treatment of the pregnant rat with the hormone, the naturally-delivered offspring have been reported to be heavier (Engfeldt and Hultquist, 1953; Campbell, Innes, and Kosterlitz, 1953; Nixon, 1954; Tuchmann-Duplessis and Mercier-Parot, 1955; Clendinnen and Eayrs, 1961), or of normal weight (Zamenhof, Mosley, and Schuller, 1966; Ray and Hochhauser, 1969), and caesarean-delivered offspring heavier (Zamenhof, 1942; Barns and Swyer, 1952; Cotes, 1954), normal (Frazer, Huggett, and Wohlzogen, 1949; in hypophysectomised mothers: Angervall and Lundin, 1962), or lighter (Tuchmann-Duplessis and Mercier-Parot, 1955).

Increases in brain weight have been reported at birth (Zamenhof, 1942; cerebral hemisphere weight: Zamenhof, Mosley, and Schuller, 1966) but not in adulthood (Gill, Reid, McClellan, and Porter, 1967), and cerebral hyperplasia at birth (Zamenhof, 1942; Zamenhof, Mosley, and Schuller, 1966), at postnatal Day 20 (Zamenhof, Mosley, and Schuller, 1966) and in adulthood (Zamenhof, 1942). Others have not found hyperplasia at 33 or 60 days (Clendinnen and Eayrs, 1961) and no increases in DNA were found in the adult brain (Gill, Reid, McClellan, and Porter, 1967). Cortical cell hypertrophy has been reported at 33 and 60 days (Clendinnen and Eayrs, 1961).

Studies which have sought to demonstrate a link between the reported structural changes and behaviour have found earlier maturation of reflexes (Clendinnen and Eayrs, 1961; Ray and Hochhauser, 1969), improvements in cortically-mediated behaviour (Clendinnen and Eayrs, 1961; Block and Essman, 1965; Ray and Hochhauser, 1969) or no differences in behaviour (Gill, Reid, McClellan, and Porter, 1967).

In two studies, prolongation of gestation has been observed (Nixon, 1954; Tuchmann-Duplessis and Mercier-Parot, 1955), and in others fetal mortality (Nixon, 1954) and a reduction in litter-size (Barns and Swyer, 1952; Campbell, Innes, and Kosterlitz, 1953) were apparent. An additional increase in body-weight of the gravid rat has been observed during the course of hormone administration (Nixon, 1954; Tuchmann-Duplessis and Mercier-Parot, 1955).

To date, twenty-three reports have appeared in the literature which have a direct bearing on the development of fetal or postnatal offspring of mother rats who have received growth hormone during pregnancy, and yet the exact effects of the treatment remain unclear and no concensus has emerged. Notwithstanding the continuing conjecture in the field, certain of the recent experiments have been widely accepted in recent reviews as evidence of a facilitative effect of growth hormone on prenatal development of the brain (e.g., Dawes, 1968; Gottleib, 1971; Vernikos-Danellis, 1972) although Hamburgh (1971) has referred to it as a "bit of unfinished business."

On balance, it seems probable that the procedure of administering growth hormone to the pregnant rat has some effect on the ontogeny of the offspring and, therefore, that further study might prove useful to an understanding of structure-function relationships, particularly those of the nervous system.

SECTION I

CHAPTER II

INTRODUCTION

In the research reviewed in the previous section it has frequently been asserted that the course of early development may be altered by treatment with growth hormone, and that such structural changes as the treatment may produce are reflected in differences in postnatal behaviour. It was pointed out, however, that the description and interpretation of the effects of this treatment have been made with an incomplete knowledge of the course of normal development. As the major concern of the present work is to assess the effects of prenatal treatment with growth hormone on fetal development, particularly on the development of the fetal brain, it is first necessary to describe normal development in some detail. In this first section of the thesis experiments are reported which are concerned with two issues: (a) the normal prenatal and postnatal development of body and brain of the rat and (b) attempts to influence the development of the fetus by the administration of growth hormone to the mother during pregnancy.

In Experiment I normal embryonic and fetal body and brain development was studied. The results obtained were of a preliminary nature but the study provided experience with the dissection, sampling, and assay procedures that were used in subsequent studies in this section.

In Experiment II a more detailed investigation of development was

made from the embryonic to the late fetal stage. The influence of prenatal treatment of the mother with bovine growth hormone on prenatal development of the embryo and fetus was investigated. The findings indicated that growth hormone may have produced a slight enhancement of development at the mid-fetal stage but no differences that could be ascribed to the treatment were found near to normal term.

In Experiment III normal development was studied from the perinatal period through the first three postnatal weeks. The influence of ovine growth hormone given daily through the last two-thirds of pregnancy, or selectively during the rapid growth phase of the fetus, was assessed. The results demonstrated that this preparation produced no change in fetal body or brain development by late gestation.

In Experiment IV the possible influence of bovine growth hormone was again assessed, this time with mothers of lower body-weight than had previously been used. Regional assay of the fetal brain in late gestation revealed no effects due to the treatment.

It should be emphasised that although normal parameters will be presented as control data, they provide new information in themselves. They are presented in conjunction with other data both to facilitate comparisons between normal and experimental groups and to preserve the conceptual development of the thesis.

Experiment I

PRELIMINARY OBSERVATIONS ON THE PRENATAL DEVELOPMENT OF THE RAT

Before any attempt could be made to assess the functional changes that might be associated with a modified brain organisation, it was necessary first to establish the conditions under which cortical hyperplasia could be reliably produced in the offspring of mothers receiving growth hormone prenatally. There are conflicting reports in the literature on this issue (Zamenhof, 1942; Clendinnen and Eayrs, 1961; Zamenhof, Mosley and Schuller, 1966). It was also of some interest how such hyperplasia might be achieved. If, as claimed, hyperplasia were present at birth (Zamenhof, 1942; Zamenhof, Mosley, and Schuller, 1966), then it might have been established: (a) by an increase in the rate of prenatal cell proliferation in the brain; (b) by an extension of the period of proliferation; (c) by a reduction in cell death (Jacobson, 1970), or by any combination of these three. To answer this question, it is necessary to consider the normal course of cell proliferation in the fetal brain.

A search of the literature revealed little information on this subject, or, indeed, on more general quantitative features of prenatal development. Evidence about the prenatal course of certain parameters

were often found to be extrapolations of only one or two prenatal measurements and some postnatal measures. Accordingly, the first experiment was an attempt to describe in more detail the progressive changes over time of the main variables of fetal development.

Before embarking on the details of the study, the general features of prenatal development, particularly those of the nervous system, will be briefly reviewed. Unless otherwise stated, the information has been obtained from Altman and Dittmer (1962). The ages given are post-fertilisation time (= copulation time minus 8 hrs).

General:--Ovulation in the rat occurs at about 0200 hrs (Everett, 1961), approximately $7\frac{1}{2}$ - $12\frac{1}{2}$ hours after the onset of estrus. Copulation normally occurs in the evening of the day of estrus, and fertilisation several hours after ovulation. Implantation of the blastocyst into the antimesometrial border of the uterus begins on the 4th or 5th day and is complete towards the end of Day 7. The yolk sac placenta forms between Days 7-11. The true allantoic placenta begins to function around Day 11 as the embryonic blood vessels from the allantoic mesoderm grow into contact with the ectoplacental trophoblast (Huggett and Pritchard, 1945). The embryo is completely formed by Days 12-13, and the fetal stage is defined by about Day 17. Birth normally occurs at 21 days.

Brain development--The primitive streak is first apparent at about $8\frac{1}{2}$ days, and the neural groove in the neural plate at about 9 days. By Day 10, the neural tube is already partly closed. The three primordial

brain vesicles (forebrain, midbrain, and hindbrain) are evident at 10½ days, and the five divisions of the brain (telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon) by 11½ days. The cerebral hemispheres are well-defined at 12½ days at which time the cervical flexure begins. The pontine flexure follows, and at 13½ days the cerebral hemispheres begin to grow posteriorly over the diencephalon. Rapid lateral enlargement of the metencephalon occurs between Days 17-18 at which time the pontine flexure begins to straighten. In the next two days the olfactory bulbs make their appearance projecting forwards from the rhinencephalon, and transverse folds appear in the cerebellum. By Day 21, the fetal brain has acquired all the major anatomical features of the adult brain, and, by weight, is approximately 1.5% of the adult value.

The detailed analysis of developmental changes in the chemical composition of the rat brain commenced over 60 years ago with the pioneer work of Matilda and Waldemar Koch at the Wistar Institute of Anatomy and Biology in Philadelphia. Since that time, more attention has been directed towards the study of the development of the rat brain than that of any other species (Himwich, 1962); however, it is only in recent years that attempts have been made to document serial changes in the structural development of the prenatal brain.

Koch and Koch (1913) suggested that the temporal order of rat brain ontogeny be divided into four general periods:

I. Fetal: a period of rapid cell division believed to be virtually complete at birth.

II. Birth--10 days: cytodifferentiation phase, axonal and dendritic elaboration.

III. Postnatal days 10-20: rapid growth phase involving myelination and the further elaboration of cell-processes and their connections.

IV. Postnatal days 20-210+: growth proceeds more slowly with a reduction in the rate of formation of all substances except those involved in continuing myelination.

The above scheme, which attempts to marry chronological time with developmental sequences, has much usefulness and has been adopted by several workers (see McIlwain and Bachelard, 1971). The heterochronicity of regional brain development, however, imparts an arbritrariness to this schema, and to others which demarcate "critical periods." In order to facilitate comparison between different brain regions, and between different species in brain development, an alternative schema has been proposed (see below) which has as its basis serial events in development rather than absolute time. An additional stage has been included to accommodate the period of senescence which, although an integral part of development, has received little emphasis in considerations of ontogeny:

Stage I Organogenesis and neuronal multiplication

Stage II The brain "growth spurt," including:
II(a) a maturation period of axonal and dendritic growth, glial multiplication, and myelination;
II(b) a later, but overlapping period of growth in size

Stage III The mature adult state

Stage IV Senile regression

(Davison and Dobbing, 1968, p. 258)

Proliferation period and histogenesis of cell-types:--The present discussion will be restricted to the cell-production phases of Stages I and II above, as the chief interest lies in the possibility of producing experimental increases in brain cell-numbers.

Assumptions concerning the course of cell-proliferation were initially made on the basis of nucleic acid phosphorus content of the brain (Koch and Koch, 1913). More recently, direct measurement of the amount of deoxyribonucleic acid (DNA) has been used to estimate the total number of cells in the brain or its parts. The assumptions underlying the use of DNA estimations as an index of the cell-number are as follows:

(i) complete extraction of DNA from the tissue is achieved

(ii) the amount of DNA per euploid cell is constant

(iii) brain cells are euploid.

These assumptions are for the large part satisfied. Although a small percentage (1-2%) of the total DNA is found in the mitochondria (Balazs and Cocks, 1967) and a minority of neurons are known to achieve polyploidy (see summary in Jacobson, 1970, p. 68), neither of these limitations is believed to invalidate the use of the technique.

An estimate of the total number of cell nuclei in the brain may be

obtained by dividing the total amount of DNA by the amount of DNA per euploid brain cell of the rat (6.4×10^{-12} grm, Santen and Agranoff, 1963). Whereas DNA estimations provide a useful tool for the description of cell populations and changes in them over time, they do not provide any information on cell-type, and recourse to histological methods must therefore be made when such information is required. Also, the point should be made that the augmentation of the tissue content of DNA in a given period may not necessarily reflect the true increase in number of new cells in that period as cell-death is a characteristic feature of developing tissue (see Prestige, 1970). Further information on cell-histogenesis, proliferation, and migration may be obtained through the use of the powerful new technique of autoradiography (see review by Sidman, 1970a).

The histogenesis of different cell-types over time shows considerable regional specificity in the brain although within a particular region a general pattern exists in that large neurons precede smaller ones in time of appearance, and neurons generally appear before neuroglia. The successive differentiation of different cell-types, like histogenetic cell death, may be influenced by local contingencies. The possibility has been put forward that a system of hierarchic induction may operate to determine the sequential differentiation of macroneurons, microneurons, and neuroglia (Altman, Das, and Sudarshan, 1970).

Opinions differ about the respective origins of neurons and neuroglia

(for a review see Jacobson, 1970), as it is not presently known when differential gene expression operates on the germinal neuroepithelial cells to determine the outcome of their division. Briefly, the possibilities that have been suggested are:

(i) both cell-types arise consecutively from the same germ cell origin

(ii) some germinal cells produce neurons and others produce neuroglia

(iii) the germinal cell simultaneously gives rise to both an immature neuron[1], and a glioblast which remains dormant for some time before dividing.[2]

Although it was generally believed that the brain of the newly born rat had acquired almost its full complement (94-97%) of neurons (see McIlwain and Bachelard, 1971), recent evidence from autoradiographic and histologic studies indicates that in some regions (olfactory bulb, hippocampus, and cerebellum) the majority of microneurons are established in the postnatal period (Altman and Das, 1965, 1966; Altman, 1967; Altman, 1969a).

With the possible exception of a few of these short-axon cells

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[1] Jacobson (1970) points out that, in vertebrates, the term "neuroblast" is misleading as the suffix "blast" indicates that the cell is capable of mitosis and these cells are incapable of further division, although exceptions to this general rule have been noted (Angevine, 1970).

[2] The later appearance of neuroglia has been attributed by Angevine (1970) to their longer generation cycle. Sidman (1970a) has estimated the generation cycle of brainstem and cerebellar neuroglia to be between 55-114 hrs compared with 23½ hrs for external granule neuron precursors of the cerebellar cortex.

(Altman and Das, 1966), neurogenesis is essentially complete in the cerebral cortex at birth (for references see Zamenhof and Van Marthens, 1971). However, this should not be taken to imply that the population of cells in the cerebral cortex at birth is entirely neuronal. While it is true that few glial cells will be found labelled at a later period following prenatal injections of labelled thymidine, this does not mean that glial cells are not produced in the prenatal brain. Glioblasts are capable of division and successive mitoses will result in progressive dilution of the label to a point at which the intensity of labelling is indistinguishable from background artifact. The intensity of labelling in the non-dividing neuron, however, will remain the same (Sidman, 1970a).

Spatial and temporal gradients of neuron production from the ventricular germinal neuroepithelium of the cerebral isocortex have been described in detail (Berry and Eayrs, 1963; Berry, Rogers, and Eayrs, 1964; Berry and Rogers, 1965). Although the mode of migration remains uncertain (see Jacobson, 1970; Sidman, 1970b), the "inside-out" sequence of origin, ubiquitous for the cortex, is well-documented. Neurons produced early in the production phase on prenatal Days 16 and 17, migrate to the sub-pial level and remain there, whereas those produced subsequently migrate through the previously established layer to take up more superficial laminae of the six-layered isocortex. This temporal pattern of cell production and migration is shown in Figure 1.

taken from Berry, Rogers, and Eayrs (1964).

No detailed investigation has been made to date of the quantitative aspects of prenatal changes in cell-population. Mandel and Bieth (1951) found that over 25% of the adult brain DNA value was achieved by postnatal Day 2. On the basis of assays at several points late in the prenatal period, and in the 2nd and 3rd postnatal week, Winick and Noble (1965) assumed an exponential increase in total brain DNA, continuous through birth, and levelling off at about the end of the second postnatal week.

Clearly, more extensive data on the temporal aspects of prenatal cell-production are required before any interpretation of the effects of prenatal experimental treatments can be made. The object of the first experiment was to obtain such baseline measures in the embryonic and fetal brain over the last nine days of gestation.

METHOD

Subjects:--Long-Evans virgin rats from the McMaster University colony were mated following about one week of acclimatisation to the laboratory. Vaginal lavages were conducted daily between 0900-1000 hrs. and the identification of sperm defined Day 0 of pregnancy. Males were removed on that day and females were individually housed in standard plastic cages throughout pregnancy.

Sampling:--Mothers were sacrificed on prenatal Days 13, 15, 17, 19, and 21 by giving double the anaesthetic dose of "Nembutal" (sodium pentobarbitol: 100 mg/Kg/ip). Following laparotomy the uterus was exposed and the conceptuses[1] counted. Three conceptuses were removed for assay. In the case of an even number, two were taken from the cervical positions in each limb and the third from the right penultimate cervical position. For an odd number, the third conceptus was taken from the side with the greater number. Two mothers were sampled on each day. Following removal of the conceptus, the placenta and amnion were dissected away and the umbilical cord cut at its fetal[2]

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[1] "Conceptus" is used here to refer to one embryo or fetus, and its placenta and amnion.

[2] Although the fetal stage is not reached until about Day 17, both embryological and fetal stages will henceforth be referred to as "fetal" for convenience.

end. Excess fluid and blood was removed by rapid blotting on semi-absorbent paper and the fetus was immediately weighed and placed on ice.

Brain removal:--At 13 days the brain was removed with the assistance of a dissecting microscope. No attempt was made to remove that part of the embryonic skull overlying the brain as it is no more than very thin cartilage at this stage. A rostral cut was made just superior to the optic vesicle and a caudal cut through the cervical flexure (Figure 2a).

At 15 days, the embryo is about 15 mm in length. The head is well formed and flexed over onto the thorax. The cartilaginous skull was divided in the midline and peeled away laterally with forceps. The rostral cut was made superior to the lens placode and the caudal cut again at the level of the cervical flexure (Figure 2b).

At 17 days, the fetus measures about 22 mm. After removal of the overlying skull, a caudal cut was made immediately posterior to the cerebellum and the whole brain lifted clear (Figure 2c).

At 19 and 21 days, brain removal was effected without the use of the microscope. The caudal cut was made at the level of the medulla oblongata, and the whole brain including olfactory lobes was removed.

Assay procedures:--Following removal the brain was immediately immersed in ice-cold 0.2N perchloric acid in a heavy-walled centrifuge

tube which was placed in an ice-bucket. At the completion of the day's sampling, brains were homogenised by agitation with glass rod, and within one hour extraction was begun using a slight modification of Schneider's (1946) method. DNA was determined colorimetrically by Burton's modification (1956) of the diphenylamine reaction, and protein determined by the method of Lowry, Rosebrough, Farr, and Randall (1951).

RESULTS AND DISCUSSION

The purpose of the present study was to provide pilot data on prenatal development of the rat, together with experience in sampling and assay techniques.

The results are summarised in Table I. Although the sample size at each age is small, the sampling error appears to be relatively low as the changes from one day to the next appear to follow a consistent and orderly pattern. Body and brain weight both increase very rapidly over these last 9 days of gestation, and at much the same rate. The precipitous decline in the ratio brain:body (x100) provides an indication of the early disproportionate growth of the brain compared with that of the body. While protein content of the brain increases in an almost linear fashion, the augmentation of brain DNA content appears to diminish towards term. This is reflected in the estimate of cell-size (protein:DNA)[1] which increases rapidly in late gestation.

A more extensive discussion of normal development of these parameters in the rat is provided in Experiment II.

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[1]The heterogeneity of cell-types in the brain, and the additional morphological variation due to immature forms in early development, render the ratio only a very approximate estimate of average cell-size.

Table I

Normal changes in body and brain parameters of the rat from the embryonic to the late fetal stage.

Table I

Experiment I: Normal prenatal development of the rat*

Prenatal Age (days)	Wet Weight		Brain:Body (%)	Whole Brain		Protein:DNA
	Body (gm)	Brain (mg)		DNA (µg)	Protein (mg)	
13	0.078	10.6	13.7	47	0.639	13.6
15	0.256	47.2	18.5	235	1.980	8.43
17	0.836	78.4	9.5	413	4.442	10.75
19	2.311	124.4	5.6	495	8.405	16.98
21	5.069	188.2	3.7	514	14.000	27.23

*For these and for all subsequent prenatal assays, the number of samples is based on the number of mothers and not on the total number of fetuses. The value for one mother is obtained from the mean of the values for her respective fetuses. This controls for the bias due to 'litter effect' (see text).

Figure 1

Schematic representation of normal neuron production and migration in the cerebral cortex of the fetal rat over the last six days of gestation (taken from Berry, Rogers, and Eayrs, 1964).

Figure 1

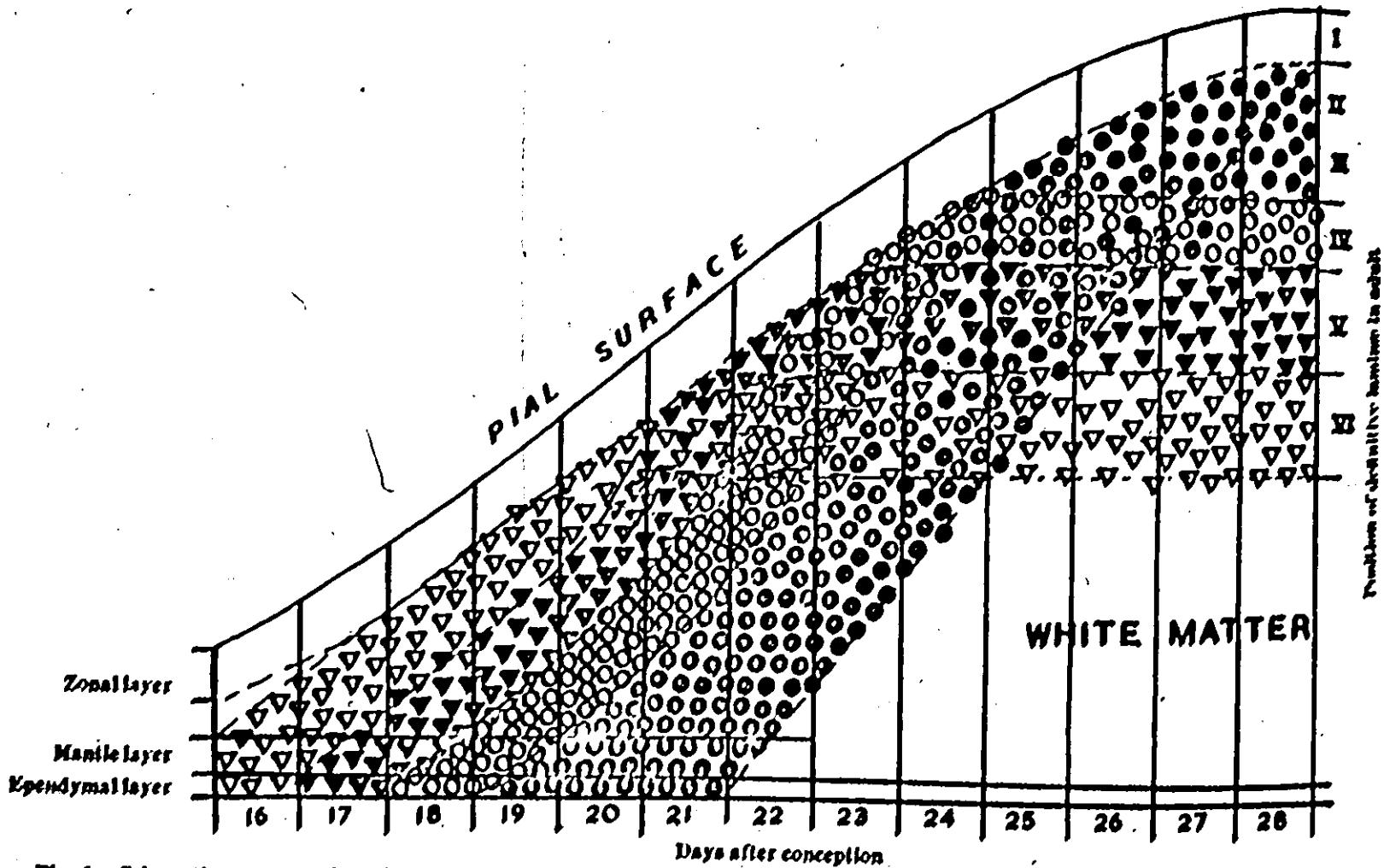

Fig. 1. Schematic representation of the positions occupied by tritium-labelled neuroblasts during successive stages of development. The signals ▽, ▼, ○, ● indicate the ages, shown along the abscissa, at which tritiated thymidine was given. The cells labelled over the period from the 19th to the 21st day of gestation formed the supragranular layers. There was no evidence of lamination into layers II and III by three-mile

Figure 2

Schematic drawings of the rat at different stages of prenatal development. The location of rostral (R) and caudal (C) cuts for brain removal are shown.

a. 13 DAY EMBRYO
b. 15 DAY FETUS
c. 17 DAY FETUS
R = ROSTRAL CUT
C = CAUDAL CUT
R
C
R
C
C

Figure 2

Experiment II

EFFECTS OF PRENATAL TREATMENT OF THE MOTHER WITH BOVINE GROWTH HORMONE

Having established the approximate developmental course of body and brain variables in the rat, in particular that of DNA accretion in the fetal brain (Experiment I), it would now be informative to examine the influence of prenatal treatment of the mother with growth hormone. The effects of such treatment have not previously been assessed during the course of prenatal development.

Berry, Rogers, and Eayrs (1964) suggested that ontogenetic development may recapitulate the phylogenetic evolution of cortical complexity in that prolongation of the period of cell-production in the ventricular neuroepithelium would result in the further addition of superficial laminae to the cerebral cortex, and the tacit proposal was subsequently made that growth hormone may act to extent the proliferation period thereby increasing the structural complexity of the cerebral cortex (Zamenhof, Mosley, and Schuller, 1966).

Under normal conditions it appears that cell-production in the fetal brain, as inferred from estimates of DNA content, begins to wane in late gestation (Experiment I). If, as claimed (Zamenhof, 1942; Zamenhof, Mosley, and Schuller, 1966), the neonatal brain is

hyperplastic at birth then it should be possible to demonstrate either a higher rate of DNA accretion, or a prolongation of the period of rapid DNA accretion, in the fetal brain. Theoretically, either effect may result not only through a stimulative action of growth hormone on cell-division but also by means of an inhibition of cell-death (see Jacobson, 1970).

The purpose of the present experiment was to investigate normal development of the rat in more detail, and to assess the possible influence on fetal development of treatment of the mother with growth hormone.

METHOD

Subjects:--In this and in all subsequent experiments subjects were Long-Evans hooded rats supplied by Blue Spruce Farms, Altamont, New York. The source of animals was changed as the McMaster Animal Breeding Unit could not supply sufficient numbers of animals at the times that they were required. The procedure for mating and maintenance of animals was the same as that described for Experiment 1. On Day 0 of pregnancy the body-weight range was 190-235 grms.

Experimental groups:--On Day 7 of pregnancy subjects were randomly assigned to either a control group (CS) or a growth-hormone group (GH). The GH group received daily subcutaneous injections of 3 USP units of growth hormone (NIH-GH-B15) contained in 0.2 ml physiological saline in the pH range 8-9. The solution concentration (15 USP units/ml) was based on the mean relative potency assay for the batch as supplied by the U.S. National Institutes of Health (NIH). The CS group received the same volume of the vehicle, in the same pH range, by the same route. Body-weights of both groups were recorded prior to injection around midday. At 1700 hrs on the day before assay, mothers received a subcutaneous injection of 0.5 mCi/Kg of a solution containing 0.5 mCi/ml $\left[^{3}H\right]$ - labelled thymidine (Amersham/Searle: TRK 300, Batch 9).

Sampling and assay procedures:--Procedures for dissection and sampling were the same as those described previously. Conceptuses were sampled from both groups on prenatal Days 13, 15, 17, 19, and 21. One day old offspring were also sampled from the control group (see Table II). After body-weight was recorded, they were decapitated and the brain removed using the technique for late gestation fetuses.

DNA and protein determinations were made as before. In addition to the measures made in the first experiment, conceptus weight, brain ribonucleic acid (RNA) content, and $\left[^{3}H\right]$ - thymidine activity in the brain were also estimated. RNA was determined by the orcinol reaction (Dische, 1955). To determine thymidine activity, an aliquot was taken from the finally extracted supernatant that would contain at least 1000 cpm of activity, and was counted in a dioxane - base scintillation medium using a Nuclear-Chicago Mark 1 liquid scintillation counter. Reliability in the counting was achieved between the second and third counts, and the third count was subsequently used for data analysis.

RESULTS

NORMAL DEVELOPMENT

The data on normal development are summarised in Tables II-IV and are presented graphically in Figures 3-8.

Conceptus weight, body weight, and brain weight:--Before Day 15, the weight of the total conceptus is less than 1 grm. Over the last six days of gestation, a rapid growth occurs resulting in a doubling of weight every two days (Figure 3). Fetal growth, by comparison, proceeds at a slightly higher rate through this period. Over the last three days of gestation, fetal weight increases by over a factor of five (Figure 4). The proportion of conceptus weight constituted by the fetus increases from approximately 25% at Day 13 to almost 85% by Day 21. From Day 13 to 17 the brain grows at a higher rate than the body but slows in comparison in the late prenatal period. The proportion of total body-weight formed by the brain drops from 16% on Day 13 to less than 4% on Day 21. Both body and brain appear to show a slight attenuation in rate of weight increase between prenatal Day 21 and postnatal Day 1 (Figure 4).

Brain protein, RNA, and DNA:--Both brain protein and brain RNA increase in approximately linear fashion at similar rates over the period studied (Figure 5). Between prenatal Day 21 and postnatal Day 1, rate

of accretion of protein drops slightly. Total brain DNA in comparison, rises at a higher rate in the early part of this period, but the rate begins to drop towards Day 21, and does not significantly change ($p > 0.1$)[1] between Day 21 and postnatal Day 1 (Figure 6). The ratio protein:DNA (an estimate of cell-size) changes little between Days 13 and 17 but as the rate of DNA increase drops in late gestation in the face of a continuing increase in protein, there is a steady increase in the ratio over the last four days of gestation (Figure 7).

$[^3H]$ - Thymidine activity:--Total $[^3H]$ - thymidine activity in the brain increases rapidly between Days 13 and 17 and shows no further increase thereafter (Table IV). Specific activity, expressed as cpm/mg brain used, is at its highest level on Day 13 and progressively drops towards Day 21 (Figure 8). No significant change in thymidine specific activity occurs between Day 19 and Day 21 ($p > 0.1$).

COMPARISON OF GROUPS

Mean maternal weights for the two groups during pregnancy are shown in Figure 9. Following five daily injections of growth hormone the GH group had gained significantly more weight than the CS group ($p < 0.05$). By Day 21 the mean difference between groups was 20 grms.

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[1]Unless otherwise stated, a two-tail test is used for Student's t test.

representing a 65% increase (over Days 1-21) in the GH group compared with a 55% increase in the CS group.

Litter-size and conceptus weight for the two groups at each gestational age are given in Table III. No significant differences were found between the groups at any stage in prenatal development.

Table IV shows body and brain weights and the ratio (%) between them over the period studied. No significant differences between groups were found at any age.

Measures of brain development are summarised in Table V. No significant differences between the groups were found in total brain RNA, protein :DNA ratio, or in the total or specific $[^3H]$ -thymidine activity at any age. Significant increases ($p < 0.05$) were found in total brain protein, and in total brain DNA and in the GH group compared with the CS group on Day 17, on Day 19 the increases bordered on significance ($0.1 > p > 0.05$), and on Day 21 there were no statistically significant differences between the two groups ($p > 0.1$).

DISCUSSION

Before discussing these data, some methodological points will be considered regarding the assessment of fetal growth. Firstly, as Barcroft (1946) has noted, weight-growth curves assume a certain homogeneity of organogenetic development although development both within and between organs is structurally and functionally heterochronous. The fetal weight-growth curve therefore invariably masks periods of rapid organ growth at different stages of development.

Secondly, descriptions of growth are most frequently made on the basis of weight and have resulted in the use of exponential equations to describe weight increments over time, although weight per se may not be the best indicator of growth (Salmon-Legagneur, 1968). Growth may be more appropriately described by mathematical functions which take some account of body-length or size (Huggett and Widdas, 1951; Stephenson, 1962).

Due to a variety of genetic and other factors, the developing prenatal litter loses its statistical homogeneity as gestation advances. Notwithstanding inherent genetic, and maternal and nutritional influences fetal weight near term is known to be influenced by litter size[1]

[1] "Litter size" is used throughout to refer to the prenatal or postnatal number in the litter and not to the total litter mass which depends both on number and individual weight.

(King, 1915; Sontag and Munson, 1934; Benson and Morris, 1971; Smart, Adlard and Dobbing, 1972; Barr, Jensh, and Brent, 1973), sex (King, 1915; Freudenberger, 1932; Benson and Morris, 1971; Smart, Adlard, and Dobbing, 1972), intrauterine position (Barr, Jensh, and Brent, 1969, 1973) and resorption (Barr, Jensh, and Brent, 1973). Thus, the true growth of an individual fetus, or fetal organ, may depart from the average obtained from pooling representative samples from the litter. This is particularly true as gestation advances. The progressive increase in variability means that the average weight-growth curve becomes increasingly less representative of the individual. Sudden and rapid changes in the course of development are therefore smoothed out to some extent when samples are pooled from one litter. Further smoothing occurs when the average of several such samples are taken (i.e., from several mothers) and when slight errors in the estimation of age are made.

These methods of estimating growth have been largely adopted for convenience. It is considerably easier to weigh a fetus than to obtain its length, and representative sampling is clearly preferred to total sampling of the litter. In the present study, the sex distribution of samples was assumed to be random in both groups, and samples were always taken from the same positions in the uterus.

Normal development:--The relative proportions of fetal and

placental components of the conceptus change markedly over the last week of gestation. In the late embryo period, fluids, placenta, and embryo each form about one third by weight of the total conceptus, whereas, at term the fetus accounts for over 80% of the conceptus weight (Wykoff, 1971).

The first detailed description of fetal growth was made by Stotsenburg (1915). From his data, it appears that 96% of fetal weight at term was achieved in the last eight days of gestation; the weight-growth curve appears to be roughly exponential over this period but decelerates (less than 15% increment) over the last day of gestation. Several other studies have confirmed the rapid phase of fetal growth (Hain, 1932; Angulo y Gonzalez, 1932; Huggett and Widdas, 1951; Wykoff, 1971). Beaton, Beare, Heh Ryu, and McHenry (1954) reported data over a more extended period of gestation and show a four-fold increase between Days 14-22. The study by Angulo y Gonzalez (1932) which showed that increments in crown-rump length of the fetus proceeded in a more uniform fashion compared with body-weight adds some support to Barcroft's suggestion (1946) that length may be a more reliable characteristic of growth than weight. The increasing variability in fetal weight was attributed to an increasing influence of litter-size as term approached (Angulo y Gonzalez, 1932).

Although Wykoff (1971) reports that the logarithm of weight-growth of the conceptus and of the fetus is linear between Days 13 and 21, the

data from the present study indicate that the increase is only exponential between Days 15-19. At the beginning (Days 13-15) and end (Days 19-21) the rate of increase appears to be slightly less than exponential.

The increase in brain weight over the period studied is roughly exponential but shows attenuation on the first day of postnatal life. The asynchrony of prenatal brain growth in relation to total growth of the fetus is illustrated in Figure 2 (not to scale) and by a comparison of the relative proportions of brain and body. At 13 days, brain weight forms over 16% of total body weight but as gestation proceeds the proportion drops precipitously and is reduced four-fold by Day 21.

Net accretion of RNA, which represents principally the ribosomal fraction of the different species of RNA, shows considerable variability during the period studied. The general pattern of increase, however, parallels that of protein accretion and supports the notion that active synthesis of protein is sustained by the level of RNA (see Leslie and Davidson, 1951; Winick and Noble, 1965). The increases in total brain protein during the period studied are in good agreement with the data of Winick and Noble (1965). Calculation of the percent composition by weight of protein in the brain in the fetal period also provides good agreement with the data of Clouet and Gaitonde (1956) which show a slight decrease in this period.

As in Experiment I, total brain DNA shows a rapid and essentially linear increase between Days 14-19, followed by an attenuation in the late

prenatal[1] and early postnatal period. Some indication of a similar sigmoid accretion of net brain DNA is suggested in the data of Dobbing and Sands (1971) as their early prenatal, and perinatal data points fall below their fitted curve. Such changes in net DNA accretion as those described here may be obscured when a larger body of data is considered over a more extended period. Although Winick and Noble (1965) show a linear increase in the logarithm of brain DNA through the prenatal period, a plot of their untransformed data shows a roughly linear increase between prenatal Days 17-20 and little change between Days 20-21. The data of Mandel and Bieth (1951) show little change in total brain DNA in the early postnatal period.

Further evidence in support of this pattern of net DNA accretion is that DNA polymerase activity (an index of cell proliferative activity) falls at birth from a maximum value on prepartum Day 6(Brasel, Ehrenkranz, and Winick, 1970). The thymidine-incorporation data from the present study also fit since the highest levels of incorporation were found during the period of most rapid accretion of DNA.

It seems appropriate to ask whether the plateau in net DNA accretion through the perinatal period is independent of parturition, i.e., is timed to occur at a fixed time from conception, or is instead related to parturition and to a general reduction in growth at this time. It is clear that body and brain weight (see Figure 4) and total brain protein (Figure 5) all show a slightly reduced rate of increase from prenatal

[1]Net DNA content of the brain appears to be increasing exponentially in the early and mid-fetal stages (Fig. 6). Nevertheless, if a worst-fit of linearity is assumed over Days 15-19, the line passes above the upper confidence limit of the Day 21 value. Thus, there appears to be a distinct fall-off in the rate of DNA accretion in late gestation (see also Fig. 12).

Day 21 to postnatal Day 1, however, the rate of increase in total brain DNA is almost negligible at this time (Figure 6). Further it appears that the rate of DNA accretion begins to drop over prenatal Days 19-21 whereas no such effect is apparent for the other measures. It seems, then, that the decrease in rate of DNA accretion in the perinatal period may not be causally related to birth.

Its occurrence at this particular time, however, may be significant for survival in the rat. Associated with the changeover from placental to pulmonary respiration may be a period of relative anoxia which could be detrimental to ongoing energetic events at this time. The newborn rat is, even so, protected to some extent as a lowered cerebral metabolic rate and accelerated glycolysis provide high resistance to anoxia at this time (Villee and Hagerman, 1958).

Maternal body-weight:--Gross increases in body-weight of the pregnant rat were described by Slonaker (1931) and Hain (1932a) Using nulliparous rats with a starting weight of about 170 grms. Hain (1932a)found a total increase of 41% during pregnancy. Fifty-four percent of this increase occurred after the 15th day. Watts (1935) obtained similar values for nulliparous rats and found slightly reduced increases in 2nd and 3rd pregnancies. Cole and Hart (1938) reported that the increase in body-weight during pregnancy was associated with an increase in food intake of about 30% over non-pregnant rats. This increased intake remained fairly constant from about Day 2 to

mid-pregnancy and then showed a further increase in the second half of pregnancy. Ota and Yokoyama (1967a) reported an increase in food intake in the first 5-6 days of pregnancy. Food intake was maintained at this level until 2-3 days before parturition when it dropped. The increase in body-weight during pregnancy was 38%.

In the present study, the total percent increase was higher than the values given above. Percent increase from Days 1-15 was approximately equal to that from Days 15-21. The increase in grams over the latter period is completely accounted for by the growth in weight of the conceptus.

The general finding of an increase in maternal body-weight over and above that accounted for by the products of conception has raised questions concerning the nature of this increase. Some of it is clearly due to growth of the mammary glands, the greatest development of which occurs during pregnancy (Meites, 1966). However, the permanent increase that is observed by weaning indicates that a genuine growth has occurred (see later discussion, Experiment VII). Studies of protein metabolism in pregnancy have generally, but not always (see Blaxter, 1964), reported maternal storage of nitrogen. Beaton, Beare, Heh Ryu, and McHenry (1954) reported greater retention of nitrogen, crude fatty acids, and water, in the first 15 days of pregnancy. After 15 days, as the fetus begins its rapid growth phase, maternal nitrogen retention greatly increased, fat storage decreased, and there

was a continued retention of water. Increased protein content in the carcass of pregnant rats compared with non-pregnant controls has been reported (Poo, Lew, Lee, and Addis, 1940). Positive nitrogen balance during pregnancy was reported by Morse and Schmidt (1944), and during the last seven days of pregnancy, in the face of a falling food intake, by Morrison (1956). Data from a study by Menaker and Navia (1973) and the results of dietary selection studies (Richter and Barelare, 1938; Leshner, Siegel, and Collier, 1972) indicate increased protein intake through most of pregnancy.

Effects of prenatal administration of growth hormone:--The significant increases at Day 17, and marginally significant[1] increases at Day 19 in the GH-treated group over the CS group are open to several interpretations. If, as has been suggested, growth hormone is a proper stimulant for fetal growth, then we would expect differences to appear at Days 13 and 15 as the treatment with growth hormone was commenced well before this time, however, no such differences were found. It is probable that slight differences might be concealed within the limits of normal sampling error as the values of the parameters investigated are extremely low at these early stages of development. Also, if the treatment does not produce a change in time of onset of cell-proliferation

[1]If, on the basis of previous work (Zamenhof, 1942; Zamenhof, Mosley, and Schuller, 1966), the hypothesis is assumed that growth hormone facilitates fetal growth, the differences at 19 days attain statistical significance ($p < 0.05$; one-tail t-test).

and protein accretion, then a change in rate may not be expected until these processes are under way. The changes observed at Day 17 are consistent with this view. Even though the hormone treatment was continued through the period of rapid fetal development, however, the significance of the differences was marginal by Day 19, and no significant differences were found at Day 21. This might suggest that the results obtained at an earlier stage were due to a timing or sampling error. Both DNA and protein content of the brain are changing very rapidly at this time and small differences in the time at which samples were taken could be very critical.

An explanation based on a reduction in litter-size (Barns and Swyer, 1952; Cotes, 1954) cannot be invoked as no differences in litter-size were found between the groups at any stage of development. Alternatively, it might be argued that growth hormone did produce an effect on Days 17 and 19 and that failure to find any enhancement at 21 days was due to sampling error. Timing errors at this stage would be less critical, at least for DNA, due to the slower rate of increase.

Evidence that the dose-level of growth hormone was physiologically effective is provided by our observation of a significant increase in maternal body-weight in the GH group over that of the CS group, although the threshold for growth hormone action on the mother may be lower than that required to stimulate fetal growth. However, the dose-level of growth hormone was comparable to that used in other studies

in which an effect on fetal growth was reported (see Introduction).

Teel (1926) first observed an effect on maternal body-weight following daily injections of an extract from the anterior pituitary. Similar effects were also reported by Hain (1932a), and Watts (1935). Surprisingly, in only two studies conducted since "purified" growth hormone became available has this effect been reported (Nixon, 1954; Tuchmann-Duplessis and Mercier-Parot, 1955). It is clear from the results of the present study that the administration of a preparation of growth hormone, free from any substantial contamination by other pituitary hormones, does produce significant increases in maternal body-weight over and above that which normally occurs during pregnancy.

Hain (1932a) did not believe that the increases she observed could have been achieved by extra mammary growth, and suggested that increased deposition of fat could account for the effect. However, work in the non-pregnant animal (Greenbaum, 1953) has shown that growth hormone has a catabolic action on fat, and the energy so provided spares the dietary proteins and makes them available for skeletal growth. Whether this catabolic action of growth hormone could affect the normal stores of fat that are laid down in the first two weeks of pregnancy (Beaton, Beare, Heh Ryu, and McHenry, 1954) is not known. Evidence will be presented later (Experiment VIII) that the increase in maternal body-weight which results from growth-hormone treatment is, in part, due to an increase in growth as normally defined (Russell, 1966).

Some evidence also exists that part of the weight-increase may be due to a hyperplastic effect of growth hormone on mammary tissue during pregnancy (Moon, 1965). This will receive further discussion in a later section.

CONCLUSIONS

The results of this study support and extend the observations of Experiment I on the normal development of the rat. Over the last week of gestation, rapid fetal development was signified by roughly exponential increases in the weight of body and brain. Total brain protein and RNA increased in a similar fashion. Total brain DNA, which reflects the net cellularity of the brain, increased in a more sigmoid fashion through this period showing a plateau in the immediate postnatal period.

Prenatal treatment of the gravid rat with bovine growth hormone resulted in significantly higher body-weight gain throughout pregnancy. The effects of growth hormone treatment of the mother on fetal development were equivocal: the brains of Day 17 fetuses from growth hormone-treated mothers contained significantly more DNA and protein, but no significant differences with control fetuses were found at later stages of development. At no stage was any effect of growth hormone on litter-size observed. On the basis of these observations, no definite conclusion could be reached on the effects of growth hormone treatment on fetal brain development.

Figure 3

Weight-growth curve of the normal conceptus over the last nine days of gestation (data from Table III). Standard error of the mean indicated by vertical bars.

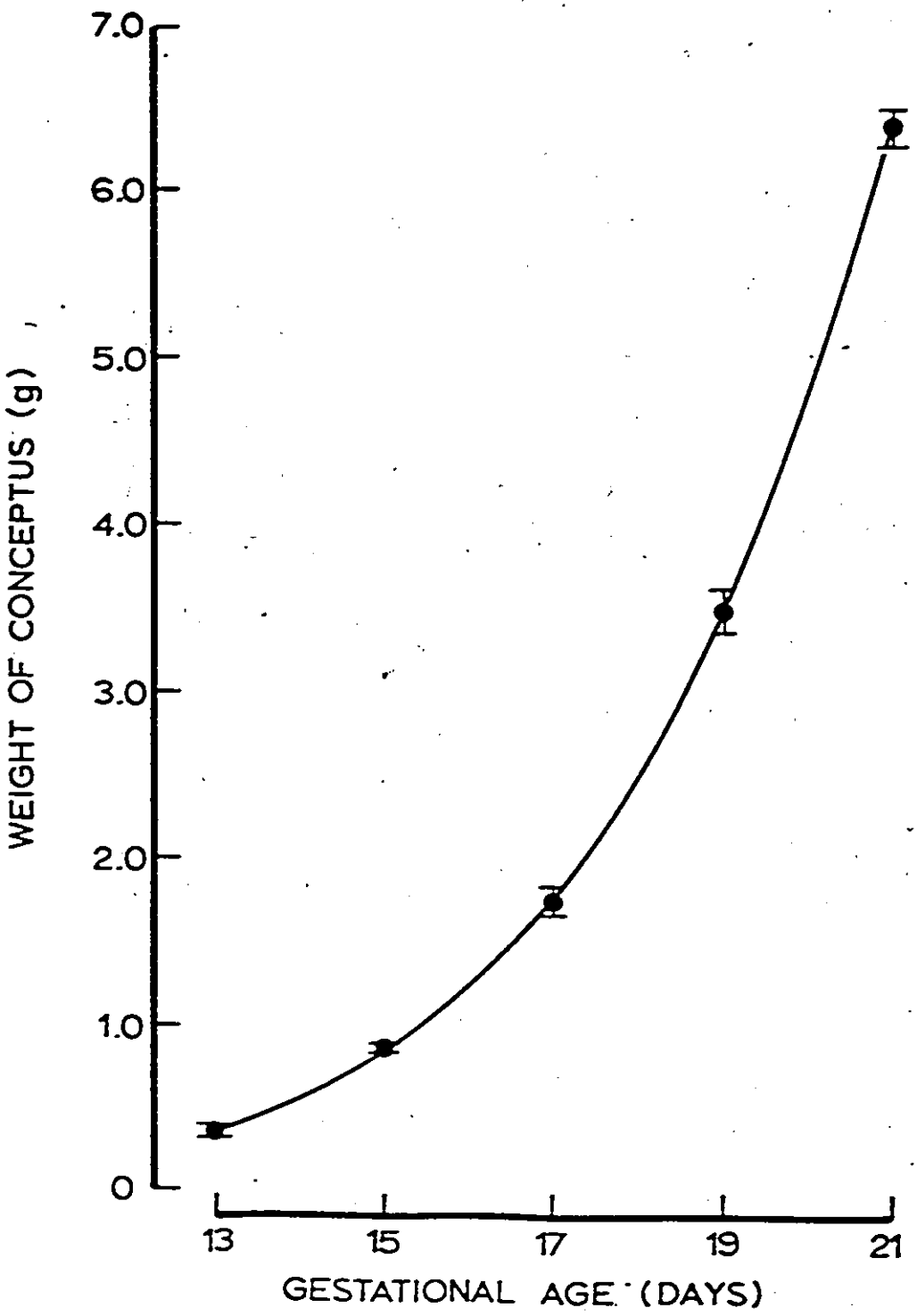
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
WEIGHT OF CONCEPTUS (g)
13
15
17
19
21
GESTATIONAL AGE (DAYS)

Figure 3

Figure 4

Weight-growth curves of normal fetus and fetal brain, and the ratio (%) between them in the prenatal and early postnatal period (data from Table IV).

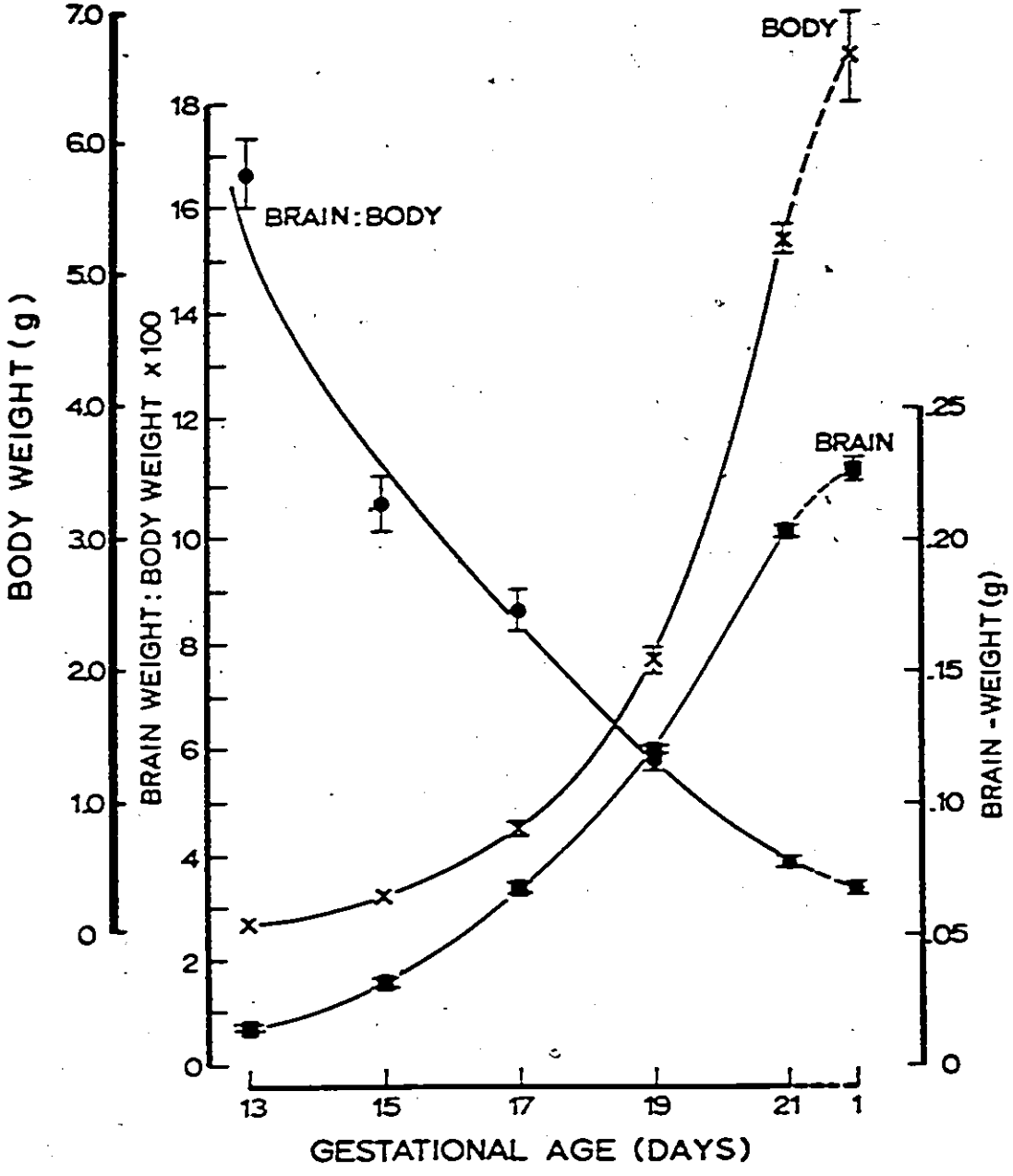
BODY WEIGHT (g)
BRAIN WEIGHT : BODY WEIGHT x 100
BRAIN - WEIGHT (g)
BODY
BRAIN
BRAIN:BODY
GESTATIONAL AGE (DAYS)
13
15
17
19
21
1

Figure 4

Figure 5

Total protein and RNA content of normal brain in prenatal and early postnatal period (data from Table V).

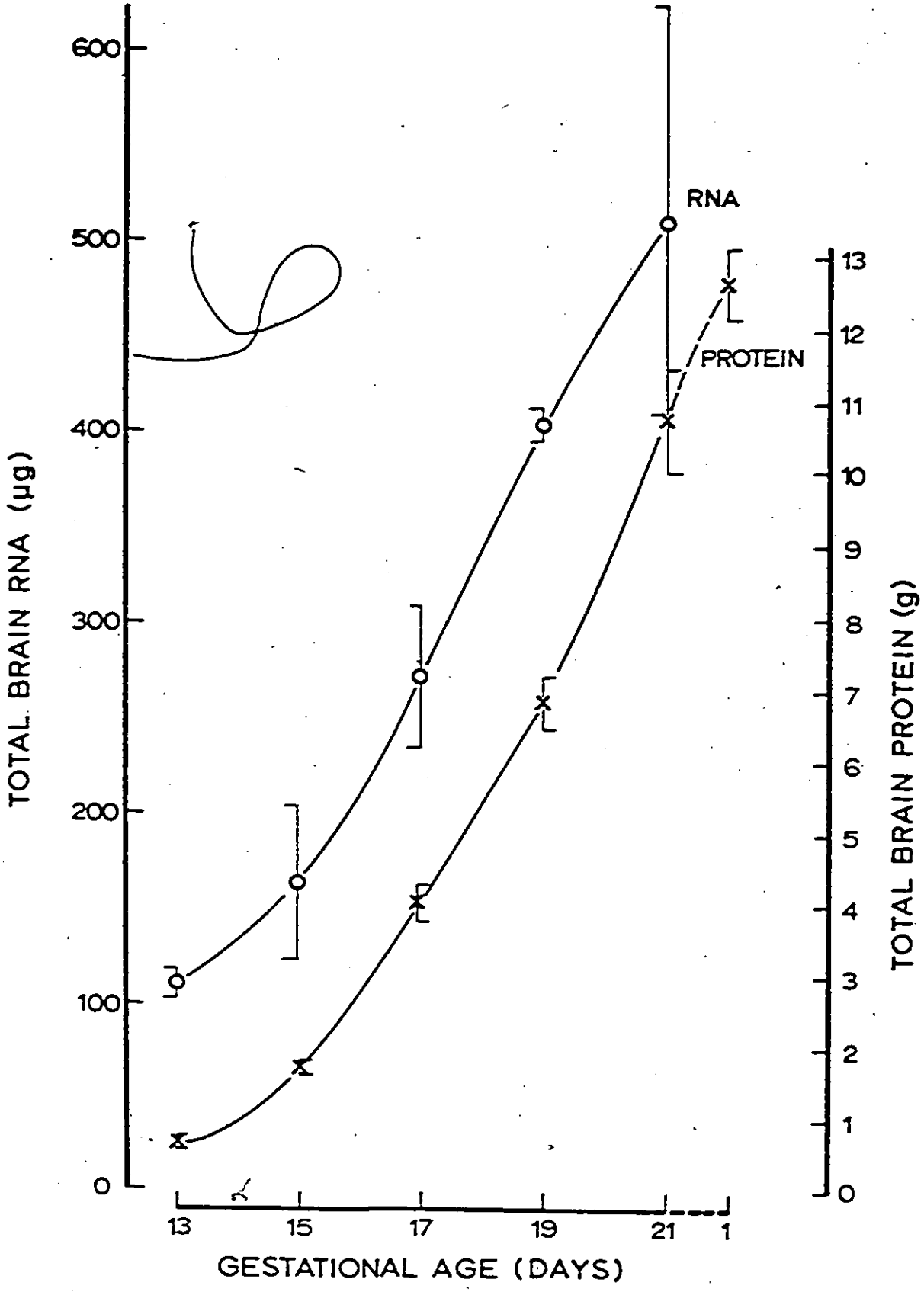
600
500
400
300
200
100
0
TOTAL BRAIN RNA (µg)
RNA
PROTEIN
13
12
11
10
9
8
7
6
5
4
3
2
1
0
TOTAL BRAIN PROTEIN (g)
13
15
17
19
21
1
GESTATIONAL AGE (DAYS)

Figure 5

Figure 6

Net change in DNA content of normal fetal brain in prenatal and early postnatal period (data from Table V).

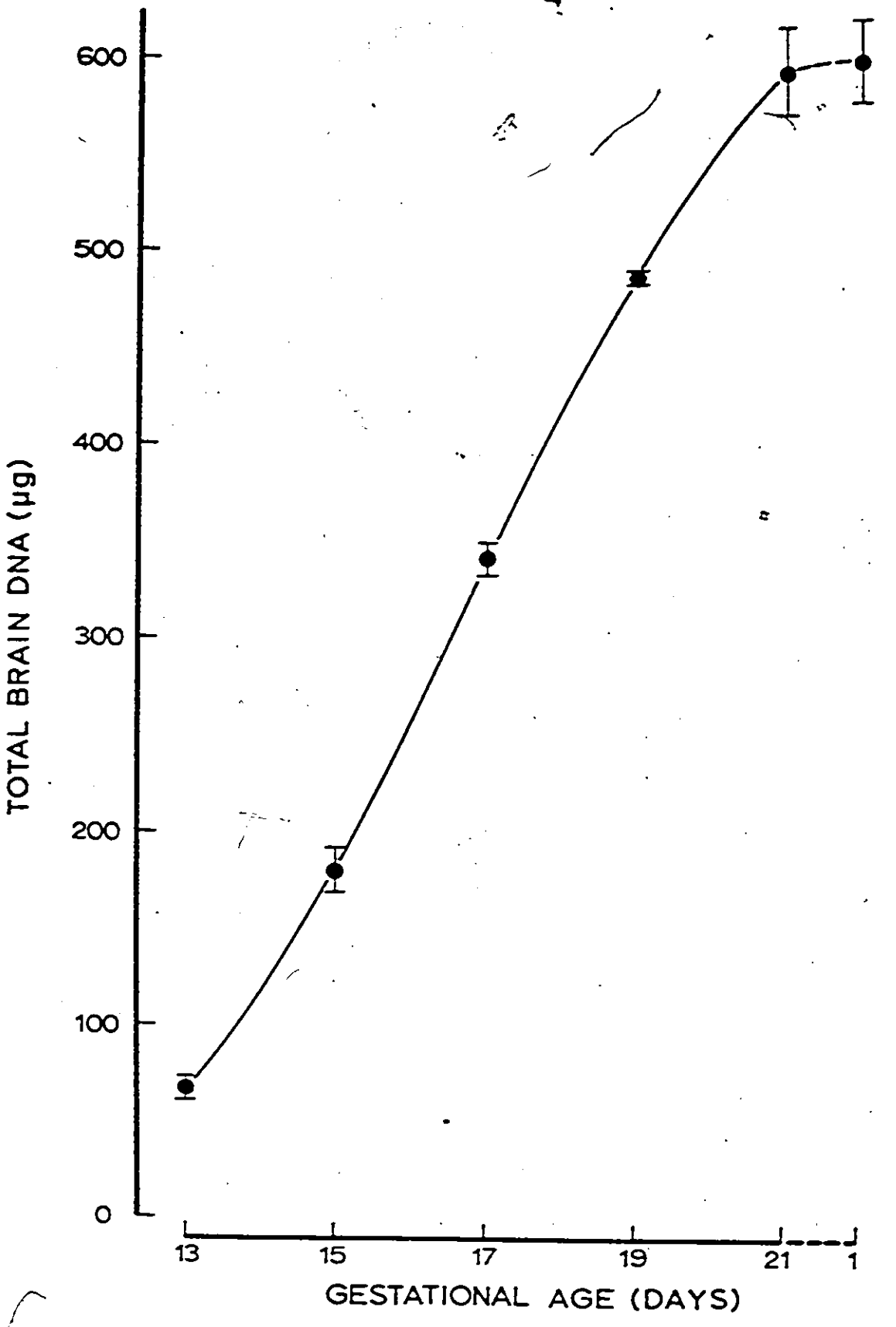
TOTAL BRAIN DNA (µg)
600
500
400
300
200
100
0
13
15
17
19
21
1
GESTATIONAL AGE (DAYS)

Figure 6

Figure 7

The ratio protein:DNA (cell-size) of normal fetal brain in the prenatal and early postnatal period (data from Table V).

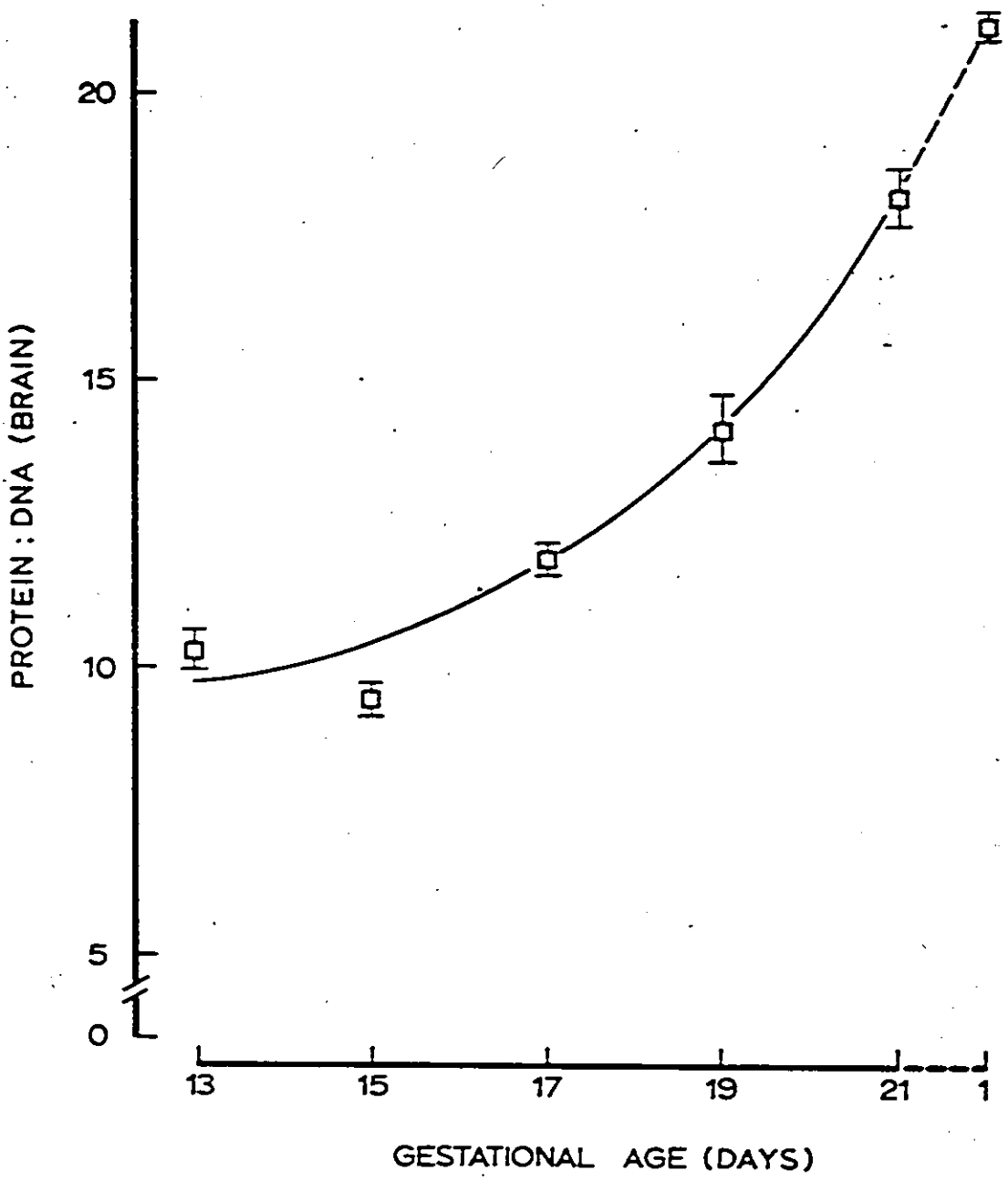
PROTEIN : DNA (BRAIN)
20
15
10
5
0
13
15
17
19
21
1
GESTATIONAL AGE (DAYS)

Figure 7

Figure 8

$[^3H]$ - thymidine specific activity in normal brain in prenatal and early postnatal period (data from Table V).

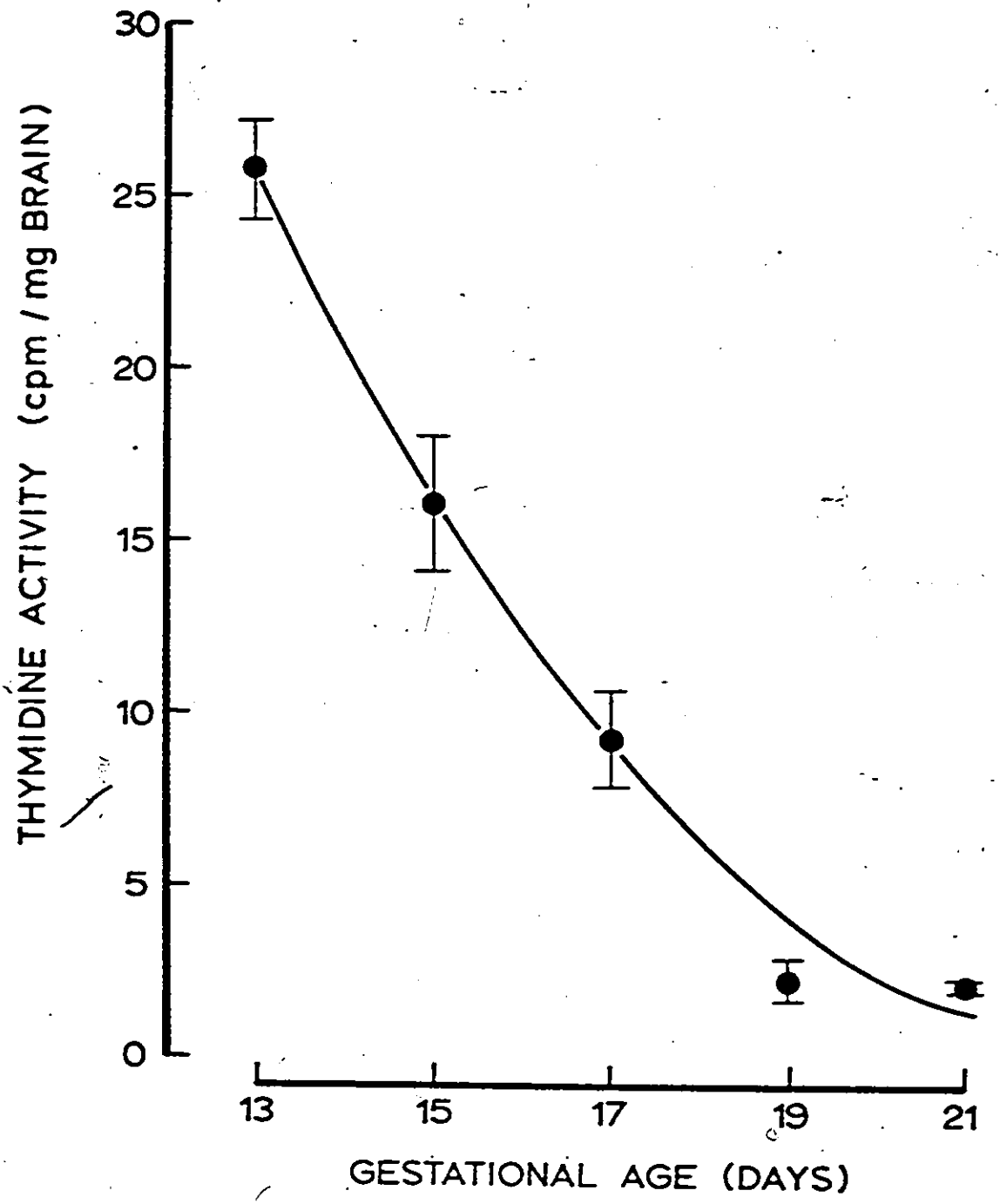
THYMIDINE ACTIVITY (cpm / mg BRAIN)
30
25
20
15
10
5
0
13
15
17
19
21
GESTATIONAL AGE (DAYS)

Figure 8

Figure 9

Body-weight gain during pregnancy for growth hormone (GH) and control (CS) mothers. From Day 12 onwards the two groups are significantly ($p < 0.05$) different from each other.

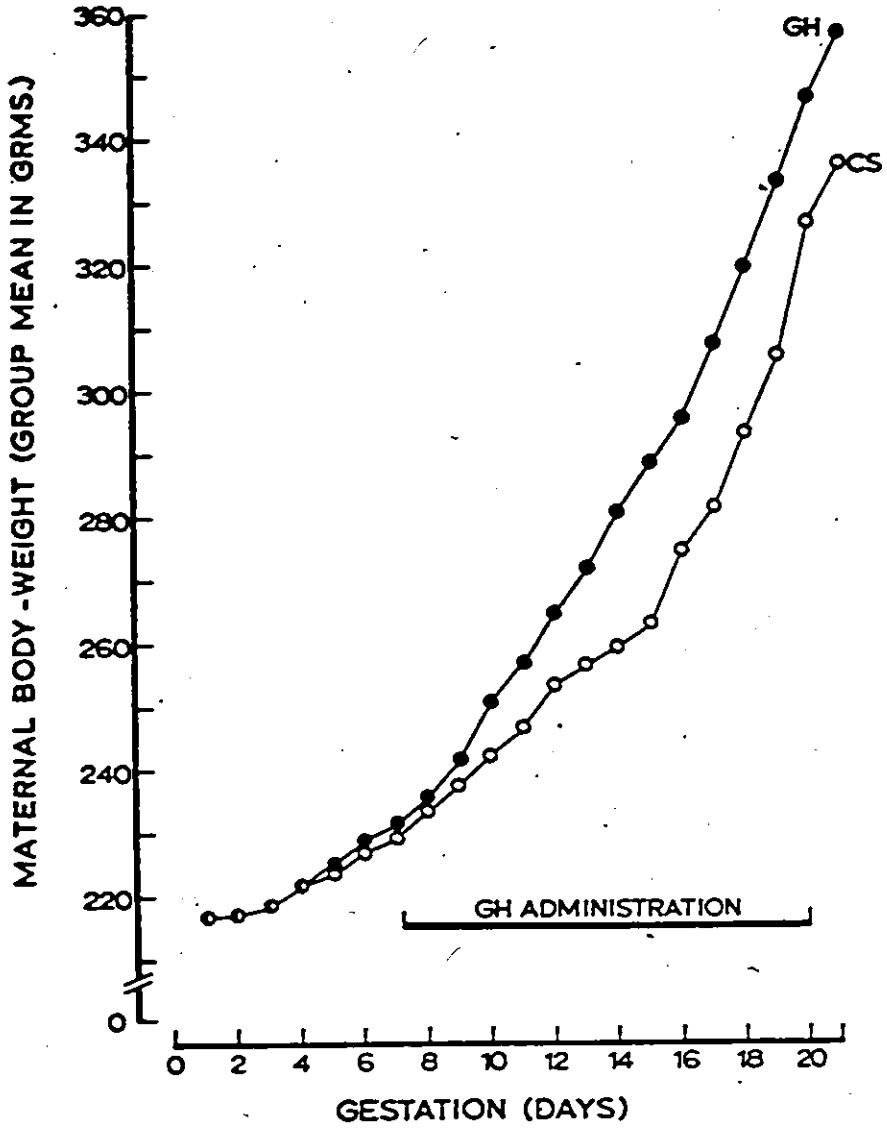
MATERNAL BODY-WEIGHT (GROUP MEAN IN GRMS.)
360
340
320
300
280
260
240
220
0
GH
CS
GH ADMINISTRATION
0 2 4 6 8 10 12 14 16 18 20
GESTATION (DAYS)

Figure 9

Table II

Number of mothers and fetuses at different stages of sampling in control (CS) and experimental (GH) groups.

Table II

Number of mothers and fetuses at different ages in control (CS) and experimental (GH) groups

		Number	
		Mothers	Fetuses
prenatal day 13	CS	3	9
	GH	3	9
prenatal day 15	CS	3	9
	GH	3	9
prenatal day 17	CS	3	9
	GH	4	12
prenatal day 19	CS	2	6
	GH	3	9
prenatal day 21	CS	3	9
	GH	3	9
postnatal day 1	CS	3	9

Table III

Litter-size and weight of conceptus at different stages of sampling for CS and GH groups. There are no significant differences ($p > 0.05$) between the groups at any stage.

Table III

Litter-Size and Weight of Conceptus at different ages for CS and GH groups

Prenatal Age (days)	Group	Means ± Standard error (S.E.)	
		Litter-Size	Conceptus weight (gm)
13	CS	12.3 ± 1.0	0.3505 ± 0.0355
	GH	12.3 ± 1.2	0.3226 ± 0.0226
15	CS	10.7 ± 1.9	0.8830 ± 0.0150
	GH	14.0 ± 1.0	0.8987 ± 0.0475
17	CS	11.7 ± 1.3	1.7480 ± 0.0871
	GH	11.5 ± 1.2	1.8813 ± 0.0832
19	CS	12.0 ± 0.0	3.4772 ± 0.1351
	GH	11.3 ± 1.5	3.7111 ± 0.0959
21	CS	10.7 ± 0.9	6.3701 ± 0.1059
	GH	11.0 ± 1.0	6.0558 ± 0.1508

Table IV

Body weight, brain weight and brain:body relationship at different stages of sampling for CS and GH groups. There are no significant differences ($p>0.05$) between the groups at any stage of development.

Table IV

Body-weight, brain-weight and brain:body relationship at different ages for CS and GH groups

Prenatal Age (days)	Group	Mean ± S.E.		
		Body-weight (gm)	Brain-weight (mg)	Brain:Body (%)
13	CS	0.0889 ± 0.0055	14.8 ± 1.0	16.72 ± 0.63
	GH	0.0784 ± 0.0036	14.8 ± 3.0	18.89 ± 3.26
15	CS	0.2906 ± 0.0077	31.3 ± 2.0	10.71 ± 0.48
	GH	0.2957 ± 0.0225	32.2 ± 2.6	10.79 ± 0.20
17	CS	0.7858 ± 0.0469	67.8 ± 1.3	8.69 ± 0.38
	GH	0.8294 ± 0.0313	73.1 ± 1.0	8.84 ± 0.22
19	CS	2.0700 ± 0.0968	119.6 ± 2.0	5.78 ± 0.15
	GH	2.1424 ± 0.0610	125.2 ± 1.0	5.87 ± 0.14
21	CS	5.2835 ± 0.1158	203.2 ± 2.0	3.86 ± 0.08
	GH	4.9040 ± 0.1537	196.3 ± 4.0	4.00 ± 0.14
Postnatal Day 1	CS	6.6780 ± 0.3500	225.7 ± 5.0	3.39 ± 0.11

Table V

Brain parameters of CS and GH groups in the prenatal and early postnatal period.

Table V

Brain Parameters of Control (CS) and Experimental (GH) Groups

Prenatal Age (days)	Group	Mean ± S.E.					
		Total Protein (mg)	Total DNA (μg)	Protein DNA	Total RNA (μg)	Total [^{3}H]-thymidine (cpm)	Specific Activity (cpm/μg)
13	CS	0.691 ± 0.072	67 ± 5.	10.3 ± 0.3	108 ± 9	3354 ± 463	257 ± 14
	GH	0.624 ± 0.151	62 ± 11.	9.8 ± 0.8	99 ± 9	2625 ± 682	193 ± 23
15	CS	1.691 ± 0.106	180 ± 11.	9.4 ± 0.3	161 ± 40	4627 ± 485	160 ± 20
	GH	1.621 ± 0.132	178 ± 13.	9.1 ± 0.1	137 ± 19	5501 ± 178	185 ± 19
17	CS	4.044 ± 0.050	341 ± 8.	11.9 ± 0.3	269 ± 37	5900 ± 723	92 ± 14
	GH	4.609 ± 0.178*	370 ± 7.	12.4 ± 0.5	308 ± 18	5612 ± 838	83 ± 10
19	CS	6.828 ± 0.342	484 ± 3.	14.1 ± 0.6	401 ± 8	2770 ± 470	24 ± 5
	GH	7.270 ± 0.108	511 ± 7.	14.3 ± 0.4	462 ± 49	3860 ± 230	32 ± 2
21	CS	10.740 ± 0.739	592 ± 25.	18.1 ± 0.5	507 ± 104	4600 ± 264	23 ± 4
	GH	10.077 ± 0.652	571 ± 27.	17.6 ± 0.9	527 ± 86	3566 ± 721	19 ± 4
Postnatal Day 1	CS	12.620 ± 0.487	597 ± 22.	21.1 ± 0.2	---	---	---

*$p < .05$, two-tail t-test.

Experiment III

THE EFFECTS OF ACUTE AND CHRONIC TREATMENT DURING PREGNANCY WITH OVINE GROWTH HORMONE, AND FURTHER OBSERVATIONS ON THE NORMAL DEVELOPMENT OF THE RAT

Experiment II provided data on the rapid growth phase of normal development of the fetal rat. The results of prenatal treatment of the mother with growth hormone, however, were equivocal; an apparent augmentation in mid-fetal development was not carried through to normal term. The inconclusiveness of these findings was possibly due to sampling or timing errors. Accordingly, it was decided to limit the period over which samples would be taken and concentrate instead on the comparison of normal and experimental fetuses in late gestation. The principal parameter of interest, brain DNA, changes little at this time and thus slight errors in timing would be less critical.

In addition, it was decided to investigate the possibility of an acute (direct or otherwise) action of growth hormone during that period when the fetus is growing rapidly and when the rate of net DNA accretion is at its highest. Such a possibility is suggested in the results of a report by Gill, Reid, McClellan, and Porter (1967). This study was considered by the authors to be a "failure to replicate" the structural (Zamenhof, 1942; Zamenhof, Mosley, and Schuller, 1966) and

behavioural (Clendinnen and Eayrs, 1961) effects of the hormone treatment although it cannot be accepted as such because the hormone was given only between prenatal Days 7-15 and was, therefore, discontinued at the commencement of the period of rapid fetal growth and DNA accretion. The authors concluded that differences in the hormone preparation might be responsible for the discrepancy, although, if an explanation were to be sought at all, it seems that the removal of a possibly acute influence of the hormone would be more likely.

In the present experiment, groups identical to those of Experiment II, at 19 and 21 days, were established using larger number of subjects. Two further groups were set up to investigate the possibility of an acute influence of growth hormone on fetal development. A "critical period" of fetal brain DNA accretion was defined as encompassing prenatal Days 16, 17, and 18. One group was injected with growth hormone from Day 7 to 15, and received saline injections on Days 16, 17, and 18 (critical period negative). A second group received saline from Day 7 to 15, and growth hormone on 16, 17, and 18 (critical period positive).

In addition to these prenatal experimental groups, some control litters were assayed in the postnatal period to extend further the data concerning normal development.

METHOD

Subjects, and mating and maintenance procedures were as for Experiment I. Maternal body-weight range on the day of conception was 185-220 grms. The hormone used was an ovine preparation (NIH-GH-S10). The reconstitution of the hormone, and its concentration and administration were as for the bovine preparation used in Experiment II.

On Day 7 of pregnancy, subjects were randomly assigned to six groups. The treatment of the various groups and their designation are summarised in Table VI. Fetuses were assayed on prenatal Days 19 and 21 only. Numbers of mothers and fetuses assayed at the two ages are given in Table VII. Three fetuses from each mother were sampled as before.

In the postnatal period, control offspring were randomly sampled from unculled litters (litter-size range: 10-15). Numbers of pups and litters at each age are given in Table XII.

Assay procedures for brain protein and brain DNA were as before.

RESULTS

Mean litter-size and maternal body-weight gain at the time of assay for the six groups are shown in Table VIII. Litter-size was not significantly different between any of the groups at either age. Maternal weight gain was computed as the percent increase in body-weight at the time of assay over the mean body-weight over Days -1, 0, and 1 (i.e., the day of conception, and the preceding and following days). In the 19 day groups, only group II (GH) showed a significant increase ($p < 0.001$) over control group I. Increases in group IV(critical period negative) were significantly greater ($p < 0.01$) than the control group by Day 15, but following cessation of the hormone treatment, the percent gain was not significantly different at 19 days (Table IX). The percent increase between Days 15 and 19 for group III (critical period positive) was comparable with that of group II during this period. The increase was greater than that of group I and bordered on statistical significance ($p.1 > p > 0.05$). Weight-gain in the 21 day GH group (group VI) was significantly greater ($p < 0.001$) than that of its associated control group (group V).

Body and brain-weight data are summarised in Table X. No statistically significant differences were found between the hormone-treated and respective control groups at either 19 or 21 days. Brain

parameters for all groups are summarised in Table XI. In the conditions designed to repeat Experiment II (groups I and II at 19 days, and groups V and VI at 21 days), no statistically significant differences were found between the hormone-treated groups and their respective controls in total protein or total DNA content of the brain nor in the ratio (cell-size) between the two. Group III, in which the hormone was given only during the critical period, was not significantly different from control group I. In group IV, in which the hormone treatment was discontinued through the critical period, significant increases ($p < 0.001$) in total brain protein were found. The increase in total brain DNA was marginally significant ($0.1 > p > 0.05$) and the increase in protein:DNA highly significant ($p < 0.001$) compared with the respective control values.

Postnatal data from control offspring are summarised in Tables XII and XIII, and are shown graphically in Figures 10-13. Prenatal values on Days 13, 15, and 17 (broken lines) are shown for continuity and are taken from the control group of Experiment II.

Pup body-weight increases in almost linear fashion in the first three postnatal weeks. In the first week the relative rate of brain growth parallels that of body growth and is reflected by the constant ratio between the two. In the second and third postnatal weeks brain growth slows and begins to plateau by the end of the third week. The brain:body ratio (%) drops accordingly at this time (Figure 10).

Total brain protein continues to increase rapidly in the postnatal

period (Figure 11). The rate of increase appears to be linear through most of this period. The plateau in brain DNA content in the perinatal period persists for the first few postnatal days. Thereafter, there is a rapid increase until about the end of the third week when a second plateau occurs (Figure 12).

The value of the ratio protein:DNA during this period is largely dictated by the change in total DNA as rate of protein accretion is relatively steady. Thus, through the late prenatal and early postnatal period there is a rapid increase in the ratio, whereas in the second phase of DNA accretion, it remains relatively constant (Figure 13).

DISCUSSION

Effect of growth hormone on fetal development:--The results are consistent with the findings of Experiment II in that daily treatment with growth hormone from Day 7 onwards did not produce any significant changes in parameters of fetal development measured at 19 and 21 days. Although the hormone was of a different species, evidence of a biological activity comparable to that of the bovine preparations was provided by the observation of significantly greater increases in maternal body-weight during pregnancy among the treated animals. However, the possibility remains that differences in the chemical properties of this species of hormone (see Russell, 1966) might give rise to differences in its qualitative properties that could be responsible for the failure here to demonstrate any effect on the fetus.

The failure, in group II and VI, to find any effect on the fetus of daily treatment with growth hormone does not necessarily imply that fetal growth cannot be stimulated by an excess of growth hormone. Daily injections of growth hormone from Day 7 onwards may have resulted in a suppression in the secretion of maternal pituitary growth hormone through an auto-feedback mechanism (see Krulich and McCann, 1966). This may have resulted in the fetus receiving a balance of endogenous and exogenous growth hormone equivalent in sum to the normal

endogenous level. However, the absence of any effect on fetal development in group III, in which growth hormone was given only during the phase of rapid fetal growth, argues against such a possibility; if it were possible to directly increase the rate of fetal growth through the administration of exogenous growth hormone, the conditions of group III should have been optimal as, presumably, there was less interference with the endogenous secretion of growth hormone.

The anomalous findings with group IV, in which the hormone was discontinued during the critical period, were unexpected and their significance is obscure. Although cessation of the hormone treatment may have produced an abnormal response of the maternal pituitary (Krulich and McCann, 1966), the failure by others to demonstrate an influence of the maternal pituitary on fetal growth (Campbell, Innes, and Kosterlitz, 1953; Tuchmann-Duplessis and Mercier-Parot, 1955; Jost and Picon, 1957) throws doubt on such an interpretation, although some reduction in fetal weight has been observed following maternal hypophysectomy (Knobil and Caton, 1953; Heggestad and Wells, 1965). Secondary changes in maternal metabolism may have resulted from the hormone regime and may have increased the availability of some limiting factor to the fetus, but any attempt at their description would be speculative. From an experimental point of view the results are interesting and warrant further investigation.

Normal postnatal development:--For several strains of rat, the logarithm of body-weight or body-length plotted against the recriprocal of time will yield straight lines (see Dunn, Murphy, and Rockland, 1947). The data obtained in the present experiment are in good agreement with those of Freudenberger (1932) for the Long-Evans strain.

Postnatal weight-growth curves for the brain were first described by Donaldson (1908). The period of rapid growth of the brain was considered to cease at about the time (70 days) at which further increases in brain weight were in approximate proportion to the 7th root of the body-weight. Plots of brain-weight against body-weight (Donaldson and Hatai, 1911) showed a very rapid increase in brain-weight up to about 30 grms of body-weight. Thereafter, brain-weight continued to increase at a much reduced rate up to a body-weight of 500 grms. The results of the present experiment are in close agreement with those of Freudenberger (1932).

Following its decline in the last week of gestation the ratio brain:body (%) remains steady in the first postnatal week. In the second and third postnatal weeks there begins a noticeable decline in the ratio as the rate of brain growth drops while that of the body remains steady.

The data of Koch and Koch (1913) show a rapid increase in total brain protein during the first three postnatal weeks. By Day 40 the daily increment has dropped to less than 0.5 mg/day and the total

content shows only slight further increase by 200 days. The data of Winick and Noble (1965) show a steady increase in total brain protein through the period of late gestation (Day 17 to birth). In the postnatal period, a continuing high rate of accretion was found up to about 30 days. Thereafter, the rate of accretion dropped considerably. The course of net accretion of total brain protein in the preweaning period, described in the present study., is in basic agreement with the pattern of accretion reported by Koch and Koch (1913) and by Winick and Noble (1965) over the same period.

In comparison to the steady increase in total brain protein through the last week of gestation and the first three postnatal weeks, the present data indicate that the net increase in total brain DNA is biphasic and essentially complete at the end of this period. This overall pattern of net DNA accretion receives support from a study by Brasel, Ehrenkranz, and Winick (1970). They reported a fall in percent increase in DNA in the late prenatal and early postnatal period; the most rapid rate of increase in DNA was found to occur between postnatal Days 6-10; thereafter, percent increase fell and between days 20-44 no further increases were observed. In addition, DNA polymerase activity, which provides a corroborative index of cell-division, dropped at parturition from a high value on prenatal Day 15, changed little in the first 5 postnatal days and reached a second peak around postnatal Day 10. A second drop then occurred to a plateau between Days 20 and

and 44. The data of Mandel and Bieth (1952) also show little change in total brain DNA in the early postnatal period. The cessation of cell-production in the brain, as measured by DNA content, is generally accepted to occur between the second and third postnatal week (Mandel and Bieth, 1952; Winick and Noble, 1965; Dobbing, 1968; Fish and Winick, 1969; Brasel, Ehrenkranz, and Winick, 1970; Dobbing and Sands, 1971).

Although no attempt has been made here to compare the development of the rat brain with that of other species, the very striking pattern of DNA accretion raises the question of whether this developmental feature has any generality among the species of Mammalia. Recent work on the development of the human brain suggests that this may be so. Dobbing and Sands (1970b) reported a similar biphasic increase in the DNA content of the brain. The first phase lasted from 15-20 weeks of gestation. The second commenced at about the 25th week, continued through the remainder of gestation, and was presumed to last until about the end of the second postnatal year of life.

Some evidence that cell-production occupies two distinct periods in the development of the guinea pig brain may be adduced from several studies. Although there have been no attempts at the direct quantification and timing of the first (principally neuronal) phase, histological studies indicate that the neuronal population is well established by about 40-45 days[1] (LaVelle, 1951; Peters and Flexner, 1950; Flexner, 1955)

[1]The gestation period of the guinea-pig is 65-66 days.

though some postnatal neurogenesis is known to occur (Altman and Das, 1967). It seems likely that a first proliferative phase occurs sometime before 40 days as cell nuclei are densely distributed, and lamination of the cerebral cortex already apparent, as early as 33 days (LaVelle, 1951), and at 35 days total brain DNA is already 30% of the adult value (Dobbing and Sands, 1970a). Again there appears to be a stationary phase between about Days 35-45 before a second phase of cell-proliferation (predominantly glial) occurs lasting to about birth (Dobbing and Sands, 1970a).

On the basis of the present observations, and those above on guinea-pig and man, it appears that the biphasic pattern of net DNA accretion (and thus cell turnover) may be characteristic of Mammalia. The species-specific timing of the two phases further suggests that the event of parturition is unrelated to any particular stage in the process of cell-production. It should be noted, however, that in neither the rat nor the guinea pig do any critical changes occur around parturition when there is a danger of anoxia which might hold long term consequences for energetic changes occurring rapidly at this time.

The significance of there being two distinct periods of cell-production in the developing brain is presently obscure. The generally accepted view that production of the neuroglia does not occur until the majority of neurons has been produced raises questions concerning their respective lineages and indeed whether there may exist an inductive contingency between them as Altman, Das, and Sudarshan (1970) have

suggested (see Introduction). This subject has been extensively reviewed by Jacobson (1970).

A number of factors are known to influence the course of postnatal growth and development, and these will be considered in some detail in Section II. In the present investigation of normal development, continuous data have been obtained through the preweaning period on those parameters that were investigated through the prenatal period, thereby covering Stage II of the Davison and Dobbing (1968) scheme (see p. 57, present work). Stages I and II of this scheme encompass that period during which the developing brain is most susceptible to influences which may alter its constitution and function.

To summarise:--The results of the present study show that the daily administration of ovine growth hormone to the gravid rat over the last two-thirds of pregnancy produced significantly greater increases in maternal body-weight, but had no apparent effect on litter-size or fetal development as measured on Days 19 and 21. Treatment with the hormone only during the period of rapid fetal growth was similarly without effect on fetal development by Day 19. Treatment over Days 7-15 (inclusive) resulted in significant increases in protein content, and a marginally significant increase in DNA content, of the fetal brain at 19 days. The ratio protein:DNA was also significantly increased.

Further data were obtained on parameters of normal body and brain development over the first three postnatal weeks.

Table VI

Summary of treatment of the six groups.

Table VI

Treatment of Groups

Group		Conceptual Age (days)																					
Number	Designation	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
I	Control 19	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	S	A		
II	GH 19	-	-	-	-	-	-	-	H	H	H	H	H	H	H	H	H	H	H	H	A		
III	Critical Period Positive	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	H	H	H	A		
IV	Critical Period Negative	-	-	-	-	-	-	-	H	H	H	H	H	H	H	H	H	S	S	S	A		
V	Control 21	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	A
VI	GH 21	-	-	-	-	-	-	-	H	H	H	H	H	H	H	H	H	H	H	H	H	H	A

S = saline injection

H = growth hormone injection

A = day of assay

Table VII

Summary of sampling in the six groups.

Table VII

Summary of sampling

Group	Prenatal Age	Number	
		Mothers	Fetuses
I	19	6	18
II	19	4	12
III	19	6	18
IV	19	6	18
V	21	6	18
VI	21	6	18

Table VIII

Litter-size and maternal body-weight gain in the six groups.

Table VIII

Litter Size, and gain (%) in maternal body-weight by time of assay

Group	Day of Assay	Litter-Size	Maternal Body-Weight gain (%)
I	19	10.00 ± 0.93	56.2 ± 2.4
II	19	11.00 ± 0.40	70.7 ± 0.2*
III	19	9.83 ± 1.01	56.9 ± 2.9
IV	19	10.50 ± 1.98	60.1 ± 4.3
V	21	10.83 ± 0.95	64.0 ± 2.6
VI	21	11.00 ± 1.06	89.3 ± 2.0*

*$p < 0.001$ compared with respective control

Table IX

Maternal body-weight gain before and after the critical period..

Table IX

Group		Group Body Weight		
		% increase Day 15	% increase Day 19	% change (19–15)
I	CS	34.1 ± 1.1	56.2 ± 2.4	22.05 ± 1.7
II	GH 19	43.8 ± 1.1***	70.7 ± 0.2***	26.9 ± 1.0**
III	positive	30.6 ± 2.6	56.9 ± 2.9	26.2 ± 1.9*
IV	negative	39.2 ± 1.1***	60.1 ± 4.3	20.9 ± 3.3

* $0.1 > p > 0.05$

** $p < 0.05$

*** $p < 0.01$

Table X

Fetal body-weight, brain-weight, and their ratio in the six groups.

Table X

Body-weight, brain-weight and brain:body (%)

Group	Day of Assay	Mean ± S.E.		
		Body-weight (gm)	Brain weight (gm)	Brain:Body (%)
I	19	2.456 ± 0.089	0.130 ± 0.002	5.34 ± 0.11
II	19	2.321 ± 0.043	0.128 ± 0.002	5.49 ± 0.09
III	19	2.488 ± 0.037	0.129 ± 0.001	5.18 ± 0.05
IV	19	2.296 ± 0.116	0.127 ± 0.002	5.57 ± 0.19
V	21	5.174 ± 0.166	0.193 ± 0.002	3.75 ± 0.09
VI	21	5.133 ± 0.187	0.199 ± 0.004	3.90 ± 0.07

Table XI

Fetal brain parameters for the six groups.

Table XI

Brain Parameters for all groups

Group	Day of Assay	Mean ± S.E. Total Protein (mg)	Total DNA (µg)	Protein/DNA
I	19	7.01 ± 0.17	501.16 ± 8.02	14.00 ± 0.22
II	19	7.08 ± 0.22	500.25 ± 6.45	14.15 ± 0.35
III	19	7.52 ± 0.48	502.50 ± 4.26	14.99 ± 1.00
IV	19	9.15 ± 0.14**	521.83 ± 5.48*	17.55 ± 0.34**
V	21	12.75 ± 0.29	577.5 ± 6.28	22.10 ± 0.61
VI	21	12.10 ± 0.39	579.5 ± 9.33	20.90 ± 0.69

*0.1 > p > 0.05
**p < 0.001
} compared with group I

Table XII

Normal body and brain parameters over the first three postnatal weeks.

Table XII

Postnatal Development of Control Offspring: Body and Brain Weights

Age (Days)	Number		Mean ± Standard Error		
	Mothers	Pups	Body-Weight (gm)	Brain-Weight (gm)	Brain:Body
2	2	6	7.49 ± 0.10	0.2878 ± 0.0112	3.85 ± 0.21
4	2	6	9.84 ± 0.38	0.4055 ± 0.0168	4.12 ± 0.01
6	2	6	12.54 ± 0.37	0.5409 ± 0.0116	4.33 ± 0.22
7	2	6	16.18 ± 0.58	0.6895 ± 0.0134	4.27 ± 0.07
10	2	6	22.73 ± 0.77	0.9217 ± 0.0035	4.10 ± 0.20
14	2	4	31.75 ± 2.25	1.2125 ± 0.0050	3.87 ± 0.27
18	3	6	37.75 ± 0.38	1.3606 ± 0.0233	3.60 ± 0.09
22	3	6	49.66 ± 2.24	1.4090 ± 0.0521	2.84 ± 0.06

Table XIII

Normal brain parameters over the first three postnatal weeks.

Table XIII

Postnatal Brain Development of Control Offspring

Age (days)	Mean ± Standard Error		
	Total Protein (mg)	Total DNA (ug)	Protein:DNA
2	16.495 ± 0.125	677.5 ± 7.5	24.35 ± 0.05
4	22.980 ± 1.160	664.0 ± 96.0	35.10 ± 3.30
6	30.630 ± 1.030	917.5 ± 16.5	33.40 ± 0.50
7	38.690 ± 0.260	1015.0 ± 30.5	38.70 ± 1.90
10	56.785 ± 1.685	1410.0 ± 166.5	40.70 ± 3.60
14	76.980 ± 0.700	2223.0 ± 85.0	34.70 ± 1.00
18	119.737 ± 0.521	3147.3 ± 130.3	38.19 ± 1.81
22	129.133 ± 7.123	3264.0 ± 152.9	39.57 ± 1.41

Figure 10

Prenatal and postnatal changes in body-weight, brain-weight, and their ratio, for normal animals (data from Tables IV, X, and XII).

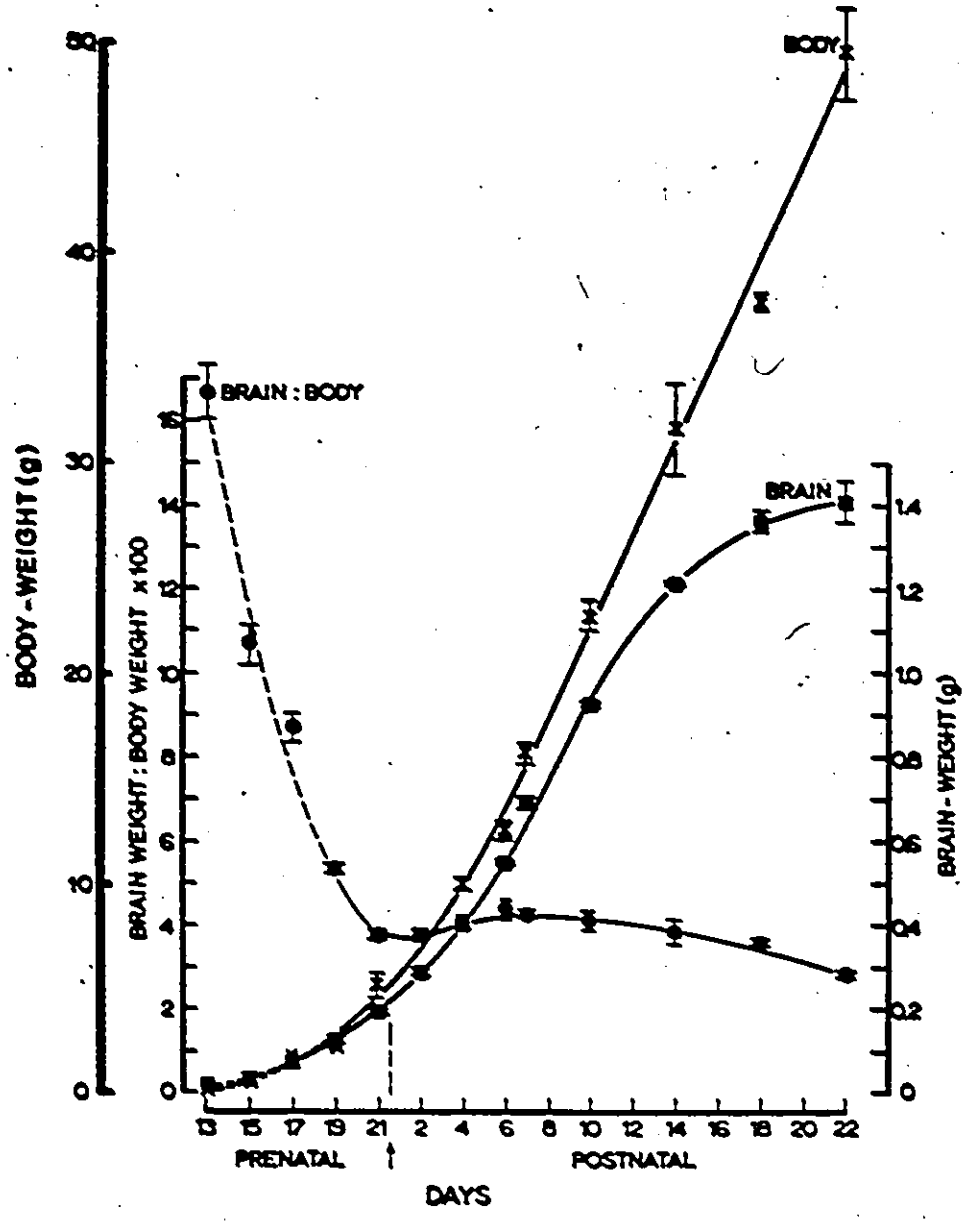
BODY
BRAIN : BODY
BRAIN
BODY - WEIGHT (g)
BRAIN WEIGHT : BODY WEIGHT x 100
BRAIN - WEIGHT (g)
PRENATAL
POSTNATAL
DAYS

Figure 10

Figure 11

Total brain protein content in the prenatal and postnatal period in normal animals (data from Tables V, XI, and XIII).

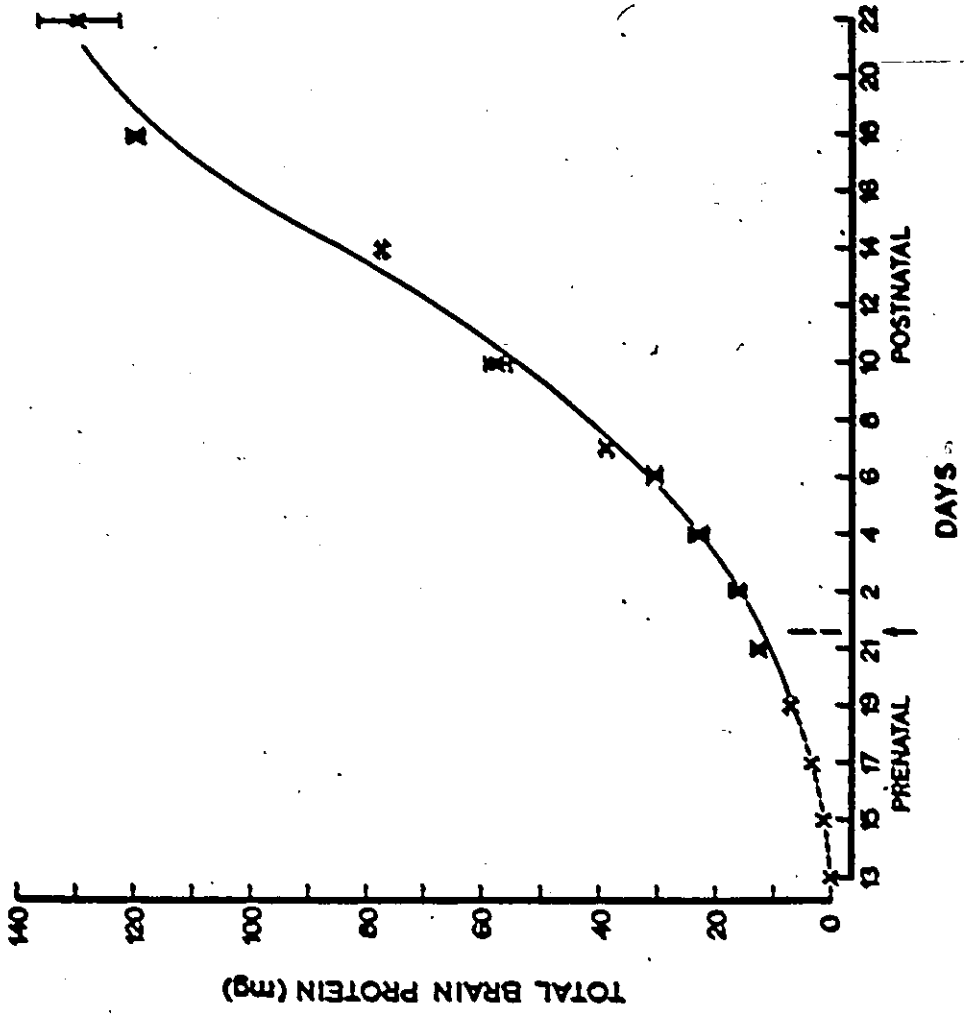
TOTAL BRAIN PROTEIN (mg)
140
120
100
80
60
40
20
0
13
15
17
19
21
1
2
4
6
8
10
12
14
16
18
20
22
PRENATAL
POSTNATAL
DAYS

Figure 11

Figure 12

Net increase in total brain DNA in the prenatal and postnatal period in normal animals (data from Tables V, XI, and XIII).

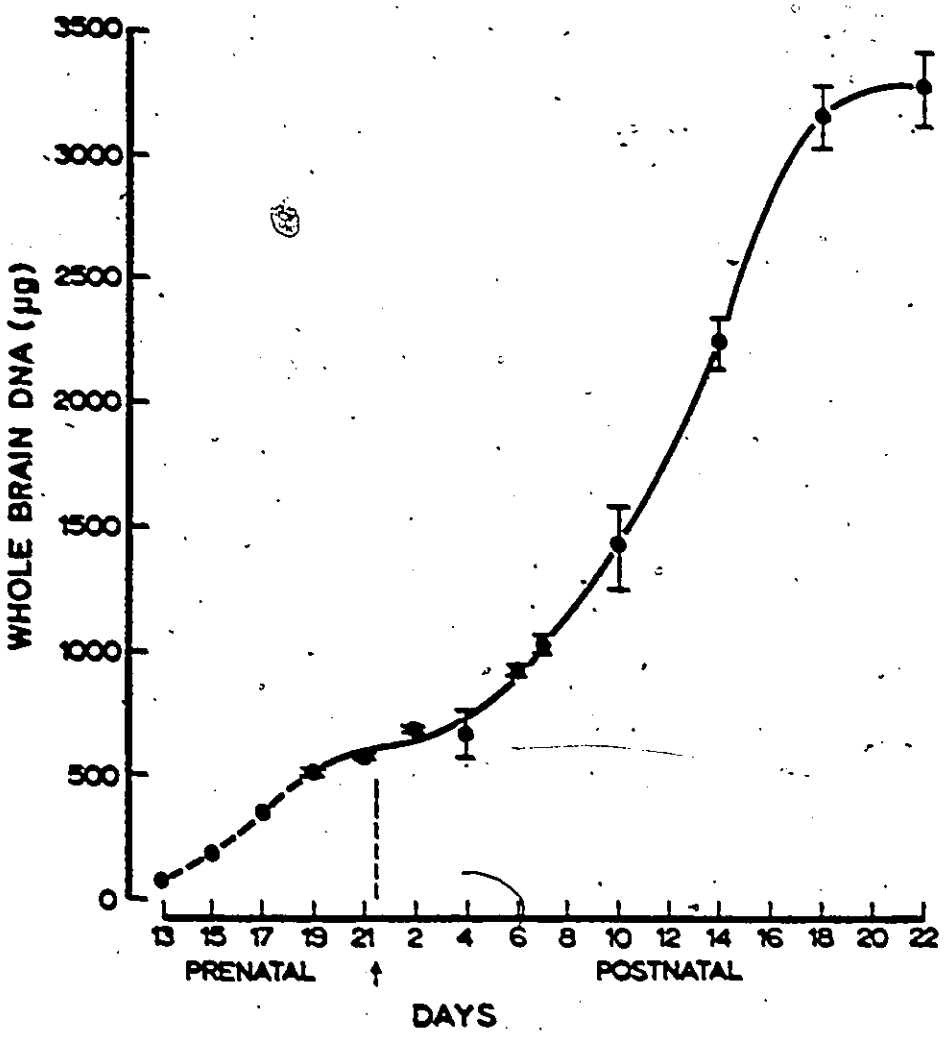
WHOLE BRAIN DNA (µg)
3500
3000
2500
2000
1500
1000
500
0
13 15 17 19 21 2 4 6 8 10 12 14 16 18 20 22
PRENATAL
POSTNATAL
DAYS

Figure 12

Figure 13

Prenatal and postnatal changes in the ratio protein:DNA in normal development (data from Tables V, XI, and XIII).

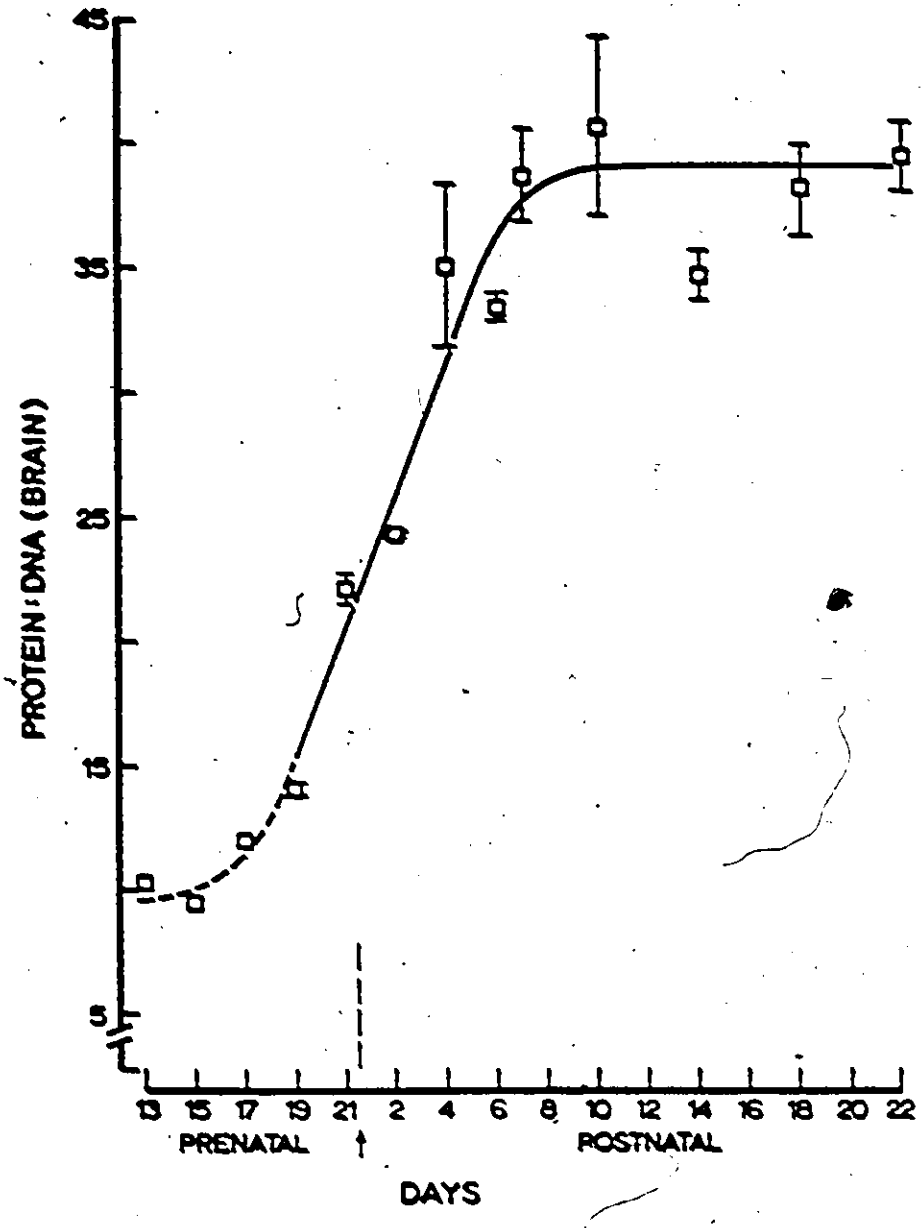
PROTEIN:DNA (BRAIN)
45
35
25
15
5
13 15 17 19 21 2 4 6 8 10 12 14 16 18 20 22
PRENATAL
POSTNATAL
DAYS

Figure 13

Experiment IV

THE EFFECTS OF PRENATAL TREATMENT WITH BOVINE GROWTH HORMONE: REGIONAL BRAIN ASSAY OF FETUSES FROM MOTHERS OF LOW BODY-WEIGHT

The results of Experiments II and III indicated that the continuous administration of growth hormone to the mother produced neither an augmentation of fetal growth nor any increment in cell-numbers in the fetal brain. Presumably, if growth hormone were capable of stimulating brain cell proliferation, its effect should have been demonstrable in a whole brain assay. However, as previous reports of growth hormone-induced hyperplasia had been restricted to the cerebral hemispheres and did not include the cerebellum or olfactory lobes (Zamenhof, 1942; Zamenhof, Mosley, and Schuller, 1966) the possibility remained that the effect may have occurred in the cerebrum and might have been masked in the whole brain assay. One further possibility was that the effect might only be demonstrable in mothers of low body-weight at the time of conception (Engfeldt and Hultquist, 1953). Also of some concern was the fact that the only unequivocal demonstration of the absence of the phenomenon (Experiment III) had been achieved using a hormone of a different species from that used by previous workers.

Having received a further supply of hormone of the original

species, it was decided to conduct a further experiment in which the three possibilities mentioned above might be investigated.

In addition to these objectives, the present study also afforded an opportunity to investigate the influence of maternal body-weight on reproductive performance by comparing the control groups in the present study with those of Experiment III. Previous reports have suggested that maternal body-weight or age may exert an influence on litter-size (Cole, 1937; Cole and Hart, 1938; Blandau and Money, 1943; Barr, Jensh, and Brent (unpublished observations), 1973), and fetal growth (Slonaker, 1912; King, 1915; Cole, 1937, Cole and Hart, 1938; Blandau and Money, 1943; Hultquist, 1950; Angervall, 1959).

METHOD

The procedures for treatment, sampling, and assay of subjects were the same as those employed in Experiment II, with the following exceptions: assays were conducted on Days 19 and 21 only; the cerebral hemispheres were weighed and assayed separately from the cerebellum and olfactory lobes (following its removal, the whole brain was placed on a glass surface and vertical cuts were made to separate the cerebral hemispheres from the olfactory lobes and from the cerebellum. The whole brain weight given in Tables XVI and XIX is obtained by adding the weights of the separate parts); the hormone used was a bovine preparation (NIH-GH-B16), and mothers were in the weight-range 140-190 grms on the day of conception. In addition to the usual measurements, placenta weights were also recorded.

A summary of the sampling of mothers and fetuses at the two gestational ages is given in Table XIV.

RESULTS

Influence of growth hormone:-- Litter-size and gain in maternal body-weight by the time of sampling for the two groups are given in Table XV. No significant differences in litter-size were found between the two groups at either 19 or 21 days. The gain (%) in body-weight at the time of assay was significantly greater in the growth hormone group both at 19 days ($p < 0.001$) and at 21 days ($p < 0.025$).

Placenta weight, fetal, body and brain weight, and the brain:body ratio are given in Table XVI. No significant differences were found between the two groups in any of these parameters at either 19 or 21 days. The results of the separate assay of the cerebrum, and cerebellum and olfactory lobes, are presented in Table XVII.[1] No significant differences were found in either weight or DNA content of the different regions between the two groups at either 19 or 21 days.

Influence of maternal body-weight:--On Day 1 of pregnancy, the females in this experiment were, on average, 17% (30 grms) lighter than those of Experiment III. All four groups were significantly lighter ($p < 0.01$) than the corresponding groups in Experiment III

[1]Protein data are not included as a large number of the samples were accidentally destroyed.

(Table XVIII). No significant differences were found in actual body-weight increase (grms) between the corresponding groups of the two experiments. Percent gain in body-weight was significantly greater in the control 19 day ($p<0.05$), control 21 day ($p<0.01$), and growth hormone 19 day ($p<0.01$) groups of this experiment compared with the corresponding groups of Experiment III.

No significant differences in litter size, body-weight, brain weight, or brain:body ratio, were found between the control groups of this experiment and the corresponding groups of Experiment III (Table XIX).

Although brain DNA data from the two experiments fell within the same range, a statistical comparison was not conducted due to slight procedural differences and changes in the purity of the standard from one experiment to the other.

DISCUSSION

These results support the findings of Experiments II and III in that the administration of growth hormone to the mother during pregnancy is without effect on the body-weight, brain-weight, or DNA content of the brain of the fetus in late gestation. Neither was there any significant effect on the weight of the placenta.

Thus, the possible objection to the results of Experiment III (in which ovine growth hormone was used) as being incomparable to those of previous workers is untenable as the present preparation was of the same species as was used in those investigations. The further objection, that growth hormone may have a selective effect on that part of the brain which acquires the majority of its neurons prenatally, is also countered as no significant effect was found when the cerebral hemispheres were analysed separately from the rest of the brain. The possibility that growth hormone may be effective when given to mothers of low body-weight also appears to be without substance, at least within the range of body-weight used here. In the present study, mean body-weight on Day 1 of pregnancy was 30 grms less than that of mother in Experiment III and yet no effect of the hormone was found.

A comparison of the normal data of the present experiment with those of Experiment III demonstrates that a significantly lower female

body-weight at the time of mating had no noticeable effect on litter-size or fetal development. Corroborative support is obtained by the observation that these lighter mothers gained as much weight during pregnancy as the heavy mothers. They therefore showed significantly greater percent gain in body-weight.

Within the range of body-weights used in these two experiments it appears that, irrespective of the breeding weight of the mother, a relatively constant amount of weight increase occurs during the course of pregnancy which is appropriate for the size of the litter being carried. This conclusion is supported by the results of Experiment VII of the present work and by a study by Pritchard and Tucker (1970) who found no differences in litter-size, or birth-weight of pups from mothers bred at 44, 63, or 82 days of age. These authors concluded that improved diet and genetic selection for laboratory stock had mitigated the effects of body-weight and age of the mother on reproductive performance that had previously been reported by others.

Such factors may also provide an explanation for the findings of Engfeldt and Hultquist (1953) who reported that the prenatal administration of growth hormone was effective in increasing the birth-weight of only those pups from mothers of low body-weight. Although the authors refer to these growth hormone-treated offspring as "giant" it appears from their data that the effect of growth hormone was to ameliorate an effect of low maternal body-weight, i.e., mothers in the low body-weight

range who were treated with growth hormone gave birth to pups which, although significantly heavier than those from control mothers in the same low range, were comparable in weight to those from control mothers in the higher weight range.

A similar therapeutic effect has been reported of prenatally administered growth hormone on the prenatal development of offspring from mothers receiving a low-calorie diet during pregnancy (Zamenhof, van Marthens, and Grauel, 1971). Here it was suggested that the action of the hormone is to mobilise maternal stores and increase the levels of circulating nutrients which, in the case of the nutritionally deprived mother (or the mother of low body-weight), may otherwise be limiting to the normal development of the fetus. In the present study, in which no maternal effects on fetal development were found, it is not surprising that the administration of growth hormone was without effect.

To summarise:--The results of the present study show that prenatal administration of bovine growth hormone to mothers of relatively low body-weight produced no significant change in placenta weight or fetal body-weight on Days 19 or 21. Neither were any differences found in the weight or DNA content of either the cerebrum, or the cerebellum and olfactory lobes, compared with controls. As in previous studies (Experiments II and III), the hormone treatment resulted in greater increases in maternal body-weight during pregnancy and did

not affect litter-size.

Normal data from the present study were compared with those of Experiment III in which average maternal body-weight was 30 grms greater at the start of pregnancy. There were no differences in absolute maternal weight gain during pregnancy but percent gain was appropriately greater in the light mothers of the present study. No differences in litter-size or fetal development were found between the two groups.

Table XIV

Summary of sampling of mothers and fetuses.

Table XIV

Experiment IV: Summary of sampling

Group	Conceptual Age	Number	
		Mothers	Fetuses
CS	19	6	18
GH	19	5	15
CS	21	7	21
GH	21	5	15

Table XV

Litter-size and maternal body-weight gain.

Table XV

Experiment IV: Litter-size and maternal body-weight gain at time of assay

Group	Day of Assay	Litter size	Maternal Body-weight (% gain)
CS	19	10.0 ± 1.1	70.4 ± 3.4
GH	19	9.2 ± 0.5	89.2 ± 2.0*
CS	21	9.9 ± 0.8	81.6 ± 3.1
GH	21	9.6 ± 0.5	102.4 ± 8.0*

* $p < 0.05$ compared with respective control value

Table XVI

Placenta weights and fetal parameters.

Table XVI

Experiment IV: Placenta, Body, and Brain weights

Group	Day of Assay	Mean Weight ± S.E.			
		Placenta (gm)	Body (gm)	Brain (gm)	Brain:Body (%)
CS	19	0.4699 ± 0.0199	2.3598 ± 0.1556	0.1233 ± 0.0041	5.2772 ± 0.1646
CH	19	0.5396 ± 0.0298	2.1768 ± 0.0718	0.1191 ± 0.0031	5.4772 ± 0.0540
CS	21	0.5110 ± 0.0256	5.2350 ± 0.1186	0.1938 ± 0.0031	3.7079 ± 0.0607
CH	21	0.5426 ± 0.0218	5.1319 ± 0.0984	0.1905 ± 0.0031	3.7128 ± 0.0317

Table XVII

Regional analysis of the brain.

Table XVII

Separate Assay of Brain Regions

Group	Mean ± S.E.			
	Cerebral Hemispheres		Cerebellum and Olfactory Lobes	
	Weight (gm)	DNA (µg)	Weight (gm)	DNA (µg)
CS 19	0.0766 ± 0.0030	362 ± 6	0.0467 ± 0.0011	123 ± 2
GH 19	0.0742 ± 0.0031	360 ± 12	0.0449 ± 0.0005	121 ± 3
CS 21	0.1292 ± 0.0022	425 ± 5	0.0646 ± 0.0011	136 ± 3
GH 21	0.1283 ± 0.0024	425 ± 4	0.0622 ± 0.0011	130 ± 4

Table XVIII

Comparison of maternal body-weight changes in Experiments III and IV.

Table XVIII

Maternal Weight Gains: Experiments III and IV

Experiment	Designation	Mean ± S.E.		
		Day 1 Body-Weight (gm)	Increase by day of assay (gm)	Gain (%)
III	CS 19	194.0 ± 1.3 **	115.0 ± 5.4	59.3 ± 2.7 *
IV	CS 19	171.3 ± 4.9	120.5 ± 6.3	70.4 ± 3.4
III	CS 21	204.8 ± 4.3 **	134.3 ± 5.0	65.8 ± 3.1 **
IV	CS 21	169.6 ± 2.2	138.1 ± 4.9	81.6 ± 3.1
III	CH 19	198.3 ± 7.3 **	148.8 ± 4.0	75.1 ± 1.0 **
IV	CH 19	163.4 ± 6.3	145.2 ± 2.6	89.2 ± 2.0
III	CH 21	196.5 ± 6.0 **	178.3 ± 6.4	90.8 ± 1.8
IV	CH 21	173.0 ± 2.3	176.6 ± 12.8	102.4 ± 8.0

*$p < 0.05$

**$p < 0.01$

Table XIX

Comparison of litter-size and fetal parameters in Experiments III and IV.

Table XIX

Litter Size and Fetal Development: Experiments III & IV

Experiment	Designation	Mean ± S.E.			
		Litter-size	Body-weight (gm)	Brain-weight (gm)	Brain:Body (%)
III	CS 19	10.00 ± 0.93	2.455 ± 0.088	0.131 ± 0.002	5.34 ± 0.11
Iv	CS 19	10.00 ± 1.10	2.359 ± 0.155	0.123 ± 0.004	5.28 ± 0.16
III	CS 21	10.83 ± 0.95	5.174 ± 0.166	0.193 ± 0.002	3.75 ± 0.09
IV	CS 21	9.86 ± 0.77	5.235 ± 0.118	0.194 ± 0.003	3.71 ± 0.06

SECTION I:
GENERAL DISCUSSION AND CONCLUSIONS

The four experiments described in this section have served two purposes. Firstly, they provide a detailed description of the embryological, fetal, and early postnatal stages in the normal development of the rat. The frequency and continuity of sampling have provided consistent and detailed data on the weight-growth curves of body and brain, and on the course of net accretion of protein and DNA in the brain throughout this period. The prenatal data fill some gaps in present knowledge concerning the early development of these parameters. The postnatal data generally support and extend the findings of others.

Secondly, these experiments have provided a detailed investigation into the effect of growth hormone, administered to the mother during the major part of pregnancy, on the development of these fetal parameters. The results have shown unequivocally that such treatment, contrary to previous reports, does not augment either fetal body growth or brain growth and does not increase the net protein or DNA content of the developing fetal brain. Neither does the treatment affect litter-size. The only significant and reliable effect of the treatment that has been observed is an increase in body-weight of the mother, during the course of administration, in excess of that which normally occurs

during the course of pregnancy.

In the light of the present findings, it would be useful to review some of the evidence bearing on the possible control of fetal growth by growth hormone produced at three sites: the fetal pituitary, the maternal pituitary, and the placenta. The following discussion will be restricted to the hormonal control of gross development of the body and will not include the effects of hormones on specific organs (e.g., control of sexual differentiation) which has been reviewed recently (Jost and Picon, 1970).

To consider first fetal growth hormone, it appears that the pituitary gland is functionally active in the fetal rat as indicated by effects on the fetal adrenals (Jost, 1966b) and fetal thyroid (Jost, 1968) following experimental apituitarism. Growth hormone has been detected in the fetal pituitary in late gestation (Contopoulos and Simpson, 1957a; Birge, Peake, Mariz, and Danghaday, 1967) although reports conflict on whether it is involved in the regulation of fetal growth or not. Raynaud and Frilley (1947) reported negligible effects on fetal growth following X-irradiation of the fetal pituitary. Decapitation of the fetus was found to result in a slight reduction in growth (Wells, 1947; Jost, 1947) but this effect has been attributed to the surgical procedure alone as some decapitates were as heavy as intact controls of the same litter (Jost, 1947). In a more recent study, decapitation of the fetus in late gestation (18 days 20 hrs) resulted in a significantly lower body

weight 67 hours later (21 days 15 hrs) compared with litter-mate controls, and it was concluded that the fetal pituitary was responsible for about 20% of fetal growth in late gestation (Heggestad and Wells, 1965). However, there is no indication that in utero position effects were controlled in the sampling procedure (see Barr, Jensh, and Brent, 1973) or that "litter effects" were considered in the statistical treatment of the data (see King, 1969; Abbey and Howard, 1973). Evidence from studies in other species (reviewed by Pecile and Muller, 1966) generally support the conclusion that prenatal growth is not under the control of the fetal pituitary.

To consider next growth hormone from the maternal pituitary, the evidence suggests that it is not a limiting factor in fetal growth. Contopoulos and Simpson (1956) found no differences between the pituitary growth hormone content (as measured by tibial assay in hypophysectomised females) of 17 and 21 day pregnant rats and non-pregnant controls. Hypophysectomy of the mother on Day 12 (Tuchmann-Duplessis and Mercier-Parot, 1955) or on Days 13 or 15 of pregnancy (Campbell, Innes, and Kosterlitz, 1953) had no effect on fetal weight, neither were any differences found between fetuses from mothers hypophysectomised on Day 12 and those from pair-fed controls (Jost and Picon, 1957). However, Knobil and Caton (1953) did find a significant reduction (14%) in body-weight of fetuses from mothers hypophysectomised on Day 12 of pregnancy compared with pair-fed controls. The reduction in

weight was associated with a significant decrease in placenta weight (6%). However, the average body-weight of both experimental and control fetuses in this study were low and other effects may have been responsible for these results (e.g., hypophysectomised rats have lower insulin output - Martin and Gagliardino, 1967). Heggestad and Wells (1965) have also reported that maternal hypophysectomy during pregnancy (Day 14) resulted in a reduction of fetal weight, although besides the difficulties already mentioned (see page 174) the effect could only be clearly demonstrated by using fetuses from unoperated mothers as controls.

Hypophysectomy of monkey mothers in pregnancy was reported to result in offspring of normal height but slightly reduced birth-weight (Smith, 1954). In man, an infant born to a hypophysectomised mother was reported to be normal (Little, Smith, Jessiman, Selenkow, van'T Hoff, Eglin, and Moore, 1958). Jackson (1955) comparing birth-weights of infants from acromegalous mothers with those of normal mothers concluded there was no difference, although his data appear to show a consistent trend towards heavier birth-weights in the acromegalous group. Saxonova (1963) has reported frequent instances of gigantism of infants from severely acromegalic mothers.

Under otherwise normal conditions, it appears that maternal growth hormone does not regulate fetal growth by way of alterations in the general metabolic environment of the fetus, although under conditions

of maternal dietary restriction exogenous growth hormone may ameliorate growth retardation of the fetus (Zamenhof, van Marthens, and Grauel, 1971) presumably by normalising the flow of nutrients from mother to fetus.

In man, evidence against a direct action of maternal growth hormone on the fetus is provided by the observation that radio-iodinated growth hormone does not cross the placenta to enter the fetal circulation (Gitlin, Kumate, and Morales, 1965; Laron, Pertzelan, Mannheimer, Goldman, and Guttman, 1966). The possibility exists that placental permeability may not be obstructive to maternal growth hormone at earlier stages in pregnancy although similar results have been obtained with rabbits (normal gestation period 31 days) in the third week of gestation (Laron, Mannheimer, and Guttman, 1966). Growth hormone is reported to cross the placenta in the mouse (see Delost, 1971) but there is no direct evidence bearing on the placental permeability to growth hormone in the rat.

Although earlier studies reported a facilitative action of exogenous growth hormone on fetal development in the rat (see review in Introduction) it appears probable that prolongation of pregnancy and other sampling errors were responsible for these effects. In the light of the hypophysectomy studies reviewed here (Campbell, Innes, and Kosterlitz, 1953; Tuchmann-Duplessis and Mercier-Parot, 1955; Jost and Picon, 1957) the possibility that other pituitary hormones in

these earlier crude preparations were by themselves, or in synergism with growth hormone, responsible for the effects appears unlikely. The results of Experiments II, III, and IV in the present study show unequivocally that the administration of purified growth hormone has no effect on fetal growth as measured in late gestation.

The finding of slightly reduced fetal growth following maternal hypophysectomy (Knobil and Caton, 1953: Heggestad and Wells, 1965) indicates that the maternal pituitary may play some role in fetal development but not a critical one. That the growth hormone content of the maternal pituitary does not increase during pregnancy (Contopoulos and Simpson, 1956) together with the results of studies in which fetal growth was unaffected by maternal hypophysectomy (Campbell, Innes, and Kosterlitz, 1953: Tuchmann-Duplessis and Mercier-Parot, 1955; Jost and Picon, 1957) suggests that maternal growth hormone is not a limiting factor in the development of the fetus, and that the fetus enjoys a relative independence of the mother with respect to the hormonal control of its growth.

If fetal growth is not under the control of its own pituitary, nor that of the mother, how then is such an orderly and systematic progression of growth maintained? Were it dependent upon the immediate availability of some major metabolite such as glucose, or indeed under some less obvious humoral control by the mother, then short-term fluctuations in these maternal parameters, e.g., resulting from

temporary exhaustion of food supply, would be expected immediately to affect the growth of the fetus and the progression of development might therefore be less consistent. Survival of the progeny would, thus, bear a more contingent relationship to the vicissitudes of the immediate environment of the mother.

The results of a study by Campbell, Innes and Kosterlitz (1965) suggest that this is not so in the rat. Reduction of food intake to one half and one third of normal over the last six days of gestation did not affect fetal or placental weights at term. Evidence from other species (see Dawes, 1968) reveals much the same thing. This suggests that the growth of the fetus is guided by some factor which buffers it against short-term changes in the mother, and which, except in extreme conditions (e.g. chronic maternal malnutrition) allows for a normal progression of prenatal growth by ensuring a more or less continuous availability of nutrients to the fetus.

The first indication of the existence of such a factor was provided by the demonstration that blood plasma from pregnant rats showed higher growth-promoting activity than did that of non-pregnant rats. Whereas plasma obtained from rats in the first five days of pregnancy had comparable growth-promoting activity to that of non-pregnant control plasma, plasma taken on Days 9-21 was three times more effective in promoting growth (Contopoulos and Simpson, 1957b). That the source of this growth hormone-like factor was not the maternal pituitary

is clear from a previous study by these authors. Extracts from pituitaries obtained on Days 17 and 21 of pregnancy were found to have comparable growth-promoting activity to the pituitaries of non-pregnant controls (Contopoulos and Simpson, 1956). Further, hypophysectomy on Day 12 of pregnancy did not reduce the growth-promoting properties of the plasma (Contopoulos and Simpson, 1959).

The first description of the principle responsible for these growth-promoting properties was achieved by Ito and Higashi (1961) and Kurosaki (1961). It resides in the syncytial portion of the fetal trophoblast (Sciarria, Kaplan, and Grumbach, 1963) and has been variously termed human placental lactogen (HPL)(Josimovich and McLaren, 1962), chorionic growth hormone prolactin (CGP) (Sciarria, Kaplan, and Grumbach, 1963), and human chorionic somatomammotropin (HCS) (Li, Grumbach, Kaplan, Josimovich, Friesen, and Catt, 1968).

A parallel relationship has been described between serum levels of this hormone and placenta growth in man, at least as far as the 36th week of gestation. Thereafter, the serum concentration of the hormone reaches a plateau while the placenta continues to increase in weight (Josimovich, Kosor, and Mintz, 1969). Bioassay of the hormone revealed a significant but low growth-promoting activity in the rat (Kaplan and Grumbach, 1964). Although its growth promoting potency is less than that of growth hormone, its concentration in maternal blood is higher and the daily production near term in man is reported to be

in the range 1.4 - 4.0 grm/day (Kaplan and Grumbach, 1965). The lactogenic properties of the hormone (Josimovich and McLaren, 1962) may be involved in the preparation for lactogenesis in late pregnancy.

The influence of the placental hormone appears to be not on the fetus but on the mother and/or the placenta. Secretion of the hormone is mainly into the mother but a small amount may also pass into the amniotic fluid (Josimovich and Attwood, 1964; Beck, Parker, and Daughaday, 1965; Kaplan and Grumbach, 1965). Thus, the fetus may orally ingest the hormone although very little is found in the fetal circulation or urine (Josimovich, Kosor, and Mintz, 1969).

Kaplan and Grumbach (1964) suggested that the placental hormone might play a critical role in the regulation of fetal metabolism and growth. The hormone acts to increase maternal fat mobilisation, diminish maternal glucose utilisation, and enhance the retention of nitrogen and minerals. Thus, by way of its placental "spokesman" the fetus is assured of a constant supply of glucose, amino acids, and minerals essential to its normal growth (Grumbach, 1971).

Such a mechanism of fetal growth control may partly explain why the alterations in maternal metabolism, that must be associated with the maternal growth response to exogenous growth hormone during pregnancy, have no effect on fetal growth. Conceivably, if the fetus receives everything that the placental hormone demands of the mother, then fetal growth may proceed normally, uninfluenced by such metabolic

changes in the mother and will only be affected when the mother cannot meet the demands of the placental hormone. Such is the case, presumably, when the diet of the mother is severely restricted. However, if it is true that exogenously administered growth hormone may ameliorate the retardation of fetal growth produced by maternal dietary restrictions (Zamenhof, van Marthens, and Grauel, 1971) then it appears that the placental hormone must at some point become ineffective in producing a further response from the mother, or the production of the placental hormone is exhausted, or that exogenous growth hormone has access to maternal reserves that the placental hormone does not.

Nevertheless, the concept of a placentomaternal unit (Grumbach, 1971) which under normal conditions acts to regulate the growth of the fetus is a very attractive one, although its role should not be considered in exclusion of the several other factors which may be involved in the elaboration of the metabolic changes of late pregnancy (Josimovich, Kosor, and Mintz, 1969). What may also be against the exclusiveness of placental hormone control is the relative continuity of rate of post-natal growth with prenatal growth (Dawes, 1968) which occurs despite the immediate cessation of chorionic influence at birth. This may not be too difficult an obstacle, however, as the rate control of postnatal growth may be assumed by a variety of factors. Perhaps it is the case that the transition from one form of control to the other(s) is responsible for the slight break in the growth curve around birth that has been

observed in several species (see Dawes, 1968).

Summary:--The body of evidence indicates that growth hormone issuing from the maternal or fetal pituitary is little involved in the regulation of fetal growth. It appears that a likely source of influence on fetal growth is exerted by a hormone from the placenta which, secreted directly into the blood circulation of the mother, ensures that the increasing metabolic needs of the fetus are met as pregnancy proceeds. These considerations by themselves do not rule out the possibility that exogenous growth hormone may specifically influence the prenatal development of the brain. The results of the experiments reported here, however, are unequivocal in this regard and provide no support for the view that fetal brain development may be enhanced through the administration of growth hormone to the mother.

However, several studies have reported early developmental gains and changes in the adult behaviour of offspring from growth hormone-treated mothers (Clendinnen and Eayrs, 1961; Block and Essman, 1965; Ray and Hochhauser, 1969). If these differences do not reside in prenatally-established structural differences in the brain, then an explanation must be sought elsewhere.

In Section II the possibility is investigated that differences in behaviour may be related to the pre-weaning experience of the offspring with their mother. An attempt will be made to demonstrate that the increase in maternal body-weight that results from the growth hormone

treatment is involved in the regulation of the interaction between mother and litter in the preweaning period. Findings will also be reported that offer an explanation for the structural changes in the neonatal brain that others have reported to result from prenatal treatment with growth hormone.

SECTION II

CHAPTER III

SECTION II

INTRODUCTION

The assumption has been made that differences observed in the early development of offspring from growth hormone-treated mothers (Clendinnen and Eayrs, 1961; Ray and Hochhauser, 1969) or in the adult behaviour of these offspring (Clendinnen and Eayrs, 1961; Block and Essman, 1965; Ray and Hochhauser, 1969) reside in structural differences in the brain. These differences have variously been reported as hyperplasia (Zamenhof, 1942; Zamenhof, Mosley, and Schuller, 1966) or hypertrophy (Clendinnen and Eayrs, 1961) of cortical neurons, and are presumed to have been established under the influence of growth hormone in the prenatal period.

The results of three experiments reported in Section I, however, demonstrated that prenatal treatment of the mother with growth hormone was ineffective in augmenting fetal body-weight, brain weight, or any of the parameters of brain development that were studied. It was concluded that growth hormone did not produce structural increments in the fetal brain.

What then are the origins of differences in postnatal behaviour that have been observed in the offspring of growth hormone-treated

mothers? Logically, it would seem that if no differences are established in the prenatal period, then they must come into existence in the postnatal period.

It has frequently been demonstrated that early experience may profoundly affect later behaviour (for reviews see: Beach and Jaynes, 1954; Levine, 1962b; Denenberg, 1964; Russell, 1971). Unfortunately, the role of the mother, in determining the early experience of the litter, has not always been considered in studies dealing with the effects of experimental manipulation in infancy on later behaviour. Beach and Jaynes (1954) pointed out the difficulty and necessity of controlling such "independent" variables in their comprehensive review of the effects of early experience, and similar cautions have continued to appear since then (Seitz, 1954; Broadhurst, 1961; Levine, 1962; Rosenblatt and Lehrman, 1963; Young, 1965; Richards, 1967; Barnett, 1968; Meier and Schutzman, 1968; Deitchman, 1970; Plaut, 1970; Russell, 1971). Others have stressed a need for a statistical treatment of the data which takes account of "litter effects" (King, 1969; Abbey and Howard, 1973). Thus, if the behaviour of a heavier mother was qualitatively or quantitatively different from that of a lighter mother, their respective litters would be subject to different experiences in the preweaning period. This, in turn, might lead to differences in physical and behavioural maturation, and perhaps to differences in behaviour in adulthood.

Seitz (1954) appears to have been the first to recognise the importance of the interactions between maternal behaviour, litter, and later behaviour of the offspring. Other studies (e.g., Young, 1965) have shown that experimental manipulation of the litter can affect the mother. Perhaps the clearest and most comprehensive statement of this problem concerning the lack of independent variable control has been made by Plaut (1970) in relation to studies of undernutrition. He points out that the interpretation of many of the studies that have been concerned with assessing the effects of undernutrition has been frequently confounded by a failure to take account of the concomitant maternal deprivation that may accompany these manipulations, and summarises the many variables that may be involved in the action between mother and pup.

The experiments reported in Section II of this thesis are principally concerned with the assessment of maternal behaviour and the effects of preweaning experience on the development and adult behaviour of the offspring. In addition to Experiments V-VIII, another study was conducted in which the performance of control offspring fostered to growth hormone-treated mothers was compared to that of growth hormone offspring fostered to control mothers in an operant learning situation involving discriminated avoidance from electric shock. Due to limitations in the design of this experiment, and principally because only few subjects could be tested in this situation, the study is not presented in the main body of the thesis but is included as an appendix.

Experiment V

PRELIMINARY STUDIES OF MATERNAL BEHAVIOUR

Since no prenatal effects of growth hormone treatment upon the fetus could be detected, and since growth hormone does not persist in the circulation long enough ($7\frac{1}{2}$ minutes in the rat - see Donovan, 1970) to exert a postnatal effect directly, an explanation of postnatal developmental precocity in the offspring must be sought in terms of changes in the mother which could be carried across parturition to influence the pups postnatally. In fact, the only consistent effect of growth hormone treatment was increased maternal body-weight and hence it was of interest to determine whether the body-weight[1] of the lactating mother influenced her nursing behaviour. The classic studies of maternal behaviour in the rat (Sturman-Hulbe and Stone, 1929; Wiesner and Sheard, 1933: Rosenblatt and Lehrman, 1963) contained no information bearing on this matter. Accordingly, two pilot studies were conducted in order to obtain an approximate profile of nursing behaviour in this strain of animals.

[1]Of the several factors which determine body-weight in the rat, the foremost is age. Other genetic, epigenetic (e.g. litter-size) and current constitutional factors being randomly distributed, it is assumed that the principal determinant of maternal body-weight here is age (see p. 224; discussion pp. 294-296).

Experiment V(a)

In this study the amount of time a mother spent with her litter during the period 0900-1200 hours was recorded daily by an observer over Days 1-28 postpartum. Each mother nursed a litter of nine containing three of her own pups and three from each of two other mothers who gave birth on the same day. Cross-fostering was conducted within 24 hours of parturition. The group of three mothers and their foster litters is referred to as a "triad" and four such triads were set up over four successive days. Mothers were individually housed with their litters in clear plastic cages containing a small amount of sawdust for nesting material. Food and water were available ad libitum from overhead hoppers. Due to re-location of the laboratory at this time, animals were housed in a temporary room which had neither heating nor lighting control. Lighting and, to some extent, heating depended upon outside conditions. Maternal body-weight was recorded at the end of each daily observation period.

Mean daily nursing times for the 12 mothers are shown in Figure 14. Following a slightly elevated value on Day 1, the average time spent nursing constituted about one half of the observation period until about the end of the third week. Thereafter, the average time progressively fell to a value of about one sixth of the observation period by the 28th day. The correlation between total nursing time for a mother over this period and her mean body-weight was not significant (product moment $r = -0.4$; $p > 0.05$).

Although the mean nursing time for the 12 mothers changed little over the first three weeks, substantial differences were noticed between the four triads. The first triad to be set up showed a low mean nursing time in the first few days of the postpartum period whereas subsequent triads showed a progressive increase in nursing time over the same period. As the mothers comprising a particular triad appeared to share no common feature other than that they gave birth at about the same time, the possibility was raised that the ambient temperature in the room may have been related to these effects, and a record of the daily temperatures throughout the period of the experiment was subsequently obtained from the Hamilton Weather Office.

Interestingly, it was discovered that over the four days on which the four triads were set up there occurred a precipitous decline in the daily temperature in the order of 25^0 F. Thus, mothers in the first triad commenced their postpartum period in temperatures of around 90^0 F while those in subsequent triads were exposed to progressively lower temperatures. Accordingly, the data were analysed by triad. The four groups of successive births are denoted triads A, B, C, and D, triad A giving birth first and triad D last. The average nursing time in the 3 hr observation period for each triad on postpartum Days 2, 3, and 4, together with the maximum daily temperature on these respective days, reveals an inverse correlational relationship between these two variables (Figure 15). Mothers exposed to high temperatures nursed

for less time than did those exposed to lower temperatures. The correlation for mean nursing time over this period with mean daily temperature was significant ($r = -0.90$, $p<0.05$).

Observations of the rats' behaviour made at the time provided some support for this chance finding. It was noted that when the room was hot mothers frequently lay outside the nest in a supine position. This is unusual for the rat and it may be that by so exposing the ventral surface cooling is more readily achieved. Observations made in subsequent studies confirm this inverse relationship between nursing time and ambient temperature. In a later discussion further evidence will be reviewed in support of this relationship and the possible role of temperature as a regulator of nursing periodicity will be considered in detail.

Having established what appeared to be a reasonable basis for the inequality of the triads, in terms of their differing experiences with ambient temperatures in the postpartum period, the relationship between maternal body-weight and nursing time was re-examined and analysed by triad (Table XX). In each of the four triads the two variables were negatively correlated. Heavy mothers spent less time with their litters than did light mothers. The statistical significance of these correlations was not assessed as there are only three observations in each group.

Experiment V(b)

In this second study maternal nursing behaviour was again observed, this time between 1500 - 2100 hrs daily. The six-hour period was shared in two-hour shifts by three observers. Ten primiparous females in the weight-range 180-250 grms were mated and insemination confirmed by vaginal lavage. They were housed under similar conditions as before except that the experiment was conducted in a thermostatically-controlled room (range: 68° - 72° F). In order to obtain data on the prepartum nesting behaviour observations were commenced on prenatal Day 15. Observations in the postpartum period were made as far as Day 15 only as, in the previous experiment, it was found that the location and integrity of the nest were not preserved beyond this time.

Mothers were divided into two groups on the basis of their mean body-weight over the 15 postpartum days. The five heaviest mothers were designated the "heavy" group (body-weight range: 292-381 grms) and the five lightest the "light" group (264-288 grms). Figure 16 shows the mean nesting time in the six-hour period for the two groups over the last six days of gestation and over the first 15 postpartum days. In late pregnancy all mothers spent a large amount of time in the proposed nest-site. No noticeable change occurred as parturition approached and no differences were apparent between the two groups.

In contrast to the results of Experiment V(a) postpartum nursing time for both groups showed a more or less steady decline through the

15 days. On 11 of the 15 days, the mean value for the heavy group was less than that for the light group but the difference was significant ($p < 0.05$) on Day 13 only. The number of complete nursing periods within the six-hour period is shown for both groups in Figure 17. Throughout the 15 days daily frequency of nursing did not show any substantial change. The mean frequency for the heavy group was greater than that of the light group on 11 of the 15 days but the difference achieved statistical significance ($p < 0.05$) on Day 9 only. The relative constancy of the frequency of nursing in the face of a steady decline in nursing-time indicates that the mean duration of a nursing period progressively decreases through the postpartum period.

Correlations of mean body-weight for the 10 mothers against their respective nursing time, or frequency of nursing, were not significant. The mean total nursing-time for the heavy group (2440 mins) was not significantly different from that for the light group (2560 mins); neither was mean total nursing frequency significantly different (heavy: 90.8, light: 81.0).

DISCUSSION AND CONCLUSIONS

The two studies described here provide conflicting evidence on the daily time characteristics of nest-time in the postpartum period. In the first study, in which the daily observation period was three hours, mean daily nest-time remained relatively steady for the first three postpartum weeks. However, in the second study, in which a six-hour observation period was used, a noticeable decline occurred over the first two weeks. These results suggested that the quantitative aspects of nesting behaviour might have a diurnal variation, such that the differences between the two studies might have been due to the time of day at which the observations were made. The results from both studies suggest an inverse relationship between maternal body-weight and nesting time but are not conclusive on this point. It is possible that such a relationship would not be manifested in a small sample observation period.

These considerations suggested that continuous recording of maternal behaviour would be desirable in order to provide full evaluation of quantitative changes in maternal behaviour in the postpartum period, and for the purposes of comparison between mothers during this time. The next study is concerned with the design of such a system for the automatic continuous recording of nest-time in the rat.

Figure 14

Experiment V(a): Nursing-time in the postpartum period (based on 3-hour daily observation period).

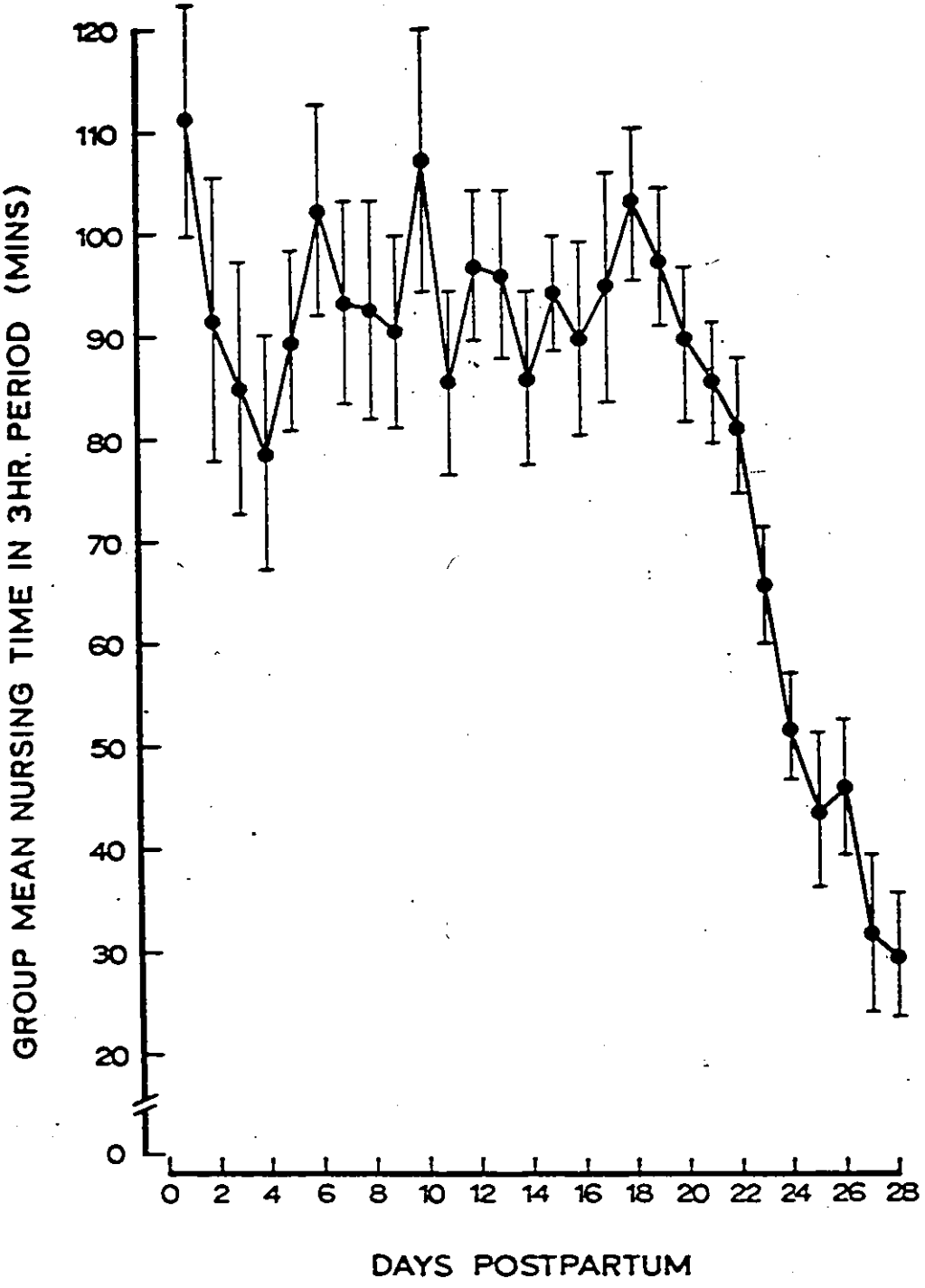
GROUP MEAN NURSING TIME IN 3HR. PERIOD (MINS)
120
110
100
90
80
70
60
50
40
30
20
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
DAYS POSTPARTUM

Figure 14

Figure 15

Experiment V(a): Nursing-time in the early postpartum period and ambient temperature.

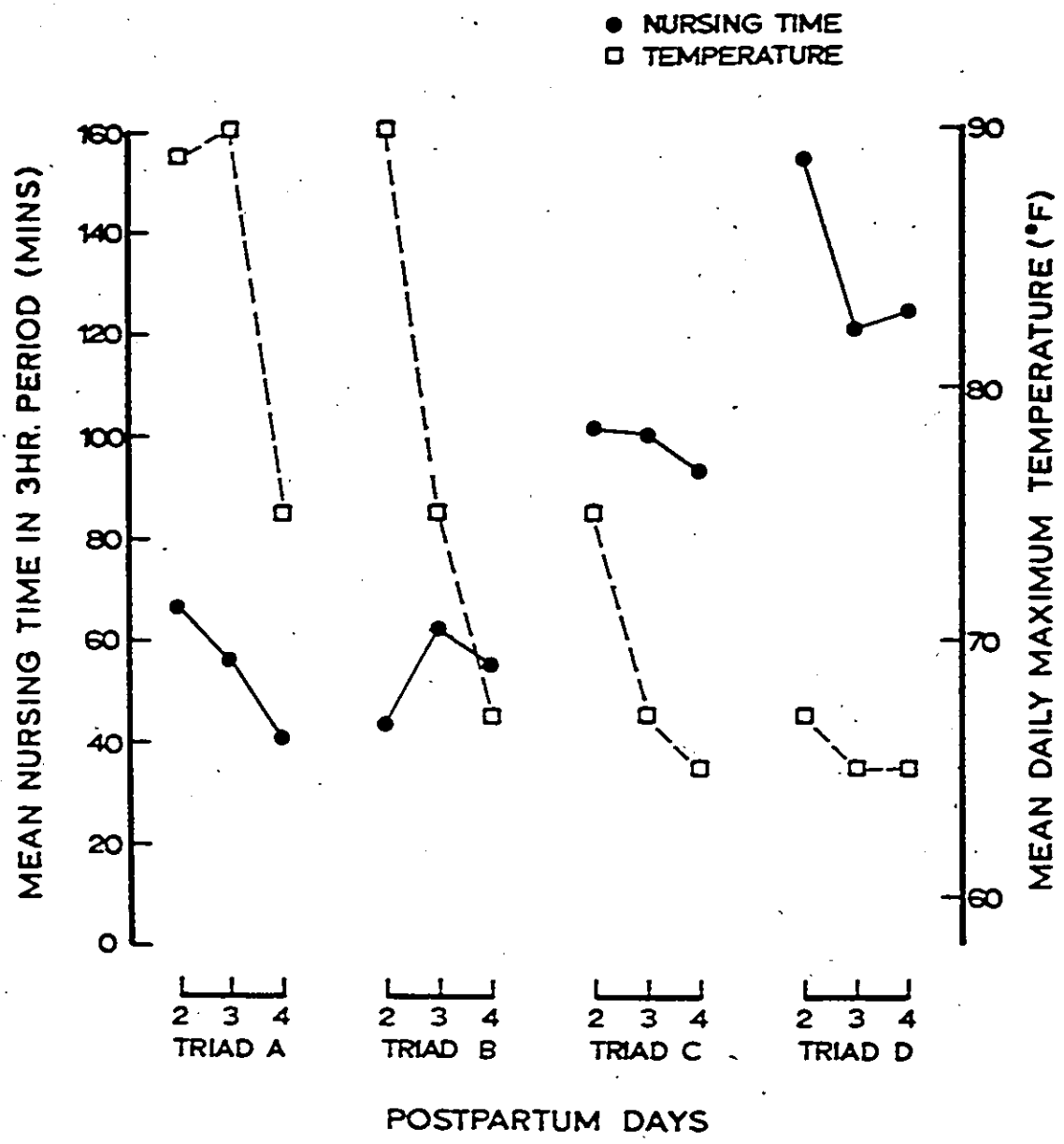
NURSING TIME
TEMPERATURE
MEAN NURSING TIME IN 3HR. PERIOD (MINS)
MEAN DAILY MAXIMUM TEMPERATURE (°F)
0
20
40
60
80
100
120
140
160
60
70
80
90
2 3 4
TRIAD A
2 3 4
TRIAD B
2 3 4
TRIAD C
2 3 4
TRIAD D
POSTPARTUM DAYS

Figure 15

Table XX

Experiment V(a): relationship between maternal body-weight and nursing-time in the postpartum period.

Table XX

Relationship between Maternal body-weight and nursing-time over 28 days

Triad	Mean body-weight of mother over 28 days (gms)	Mean nursing-time in 3 hrs over 28 days (mins)	Product Moment r
	280	61.6	
A	269	79.1	-0.56
	265	67.3	
	285	77.7	
B	274	82.6	-0.85
	261	82.7	
	284	82.3	
C	272	89.6	-0.85
	252	90.7	
	287	89.0	
D	275	90.1	-0.99
	235	95.5	

Figure 16

Experiment V(b): nest-time in late gestation and nursing-time over the first 15 postpartum days (based on 6-hour daily observation period).

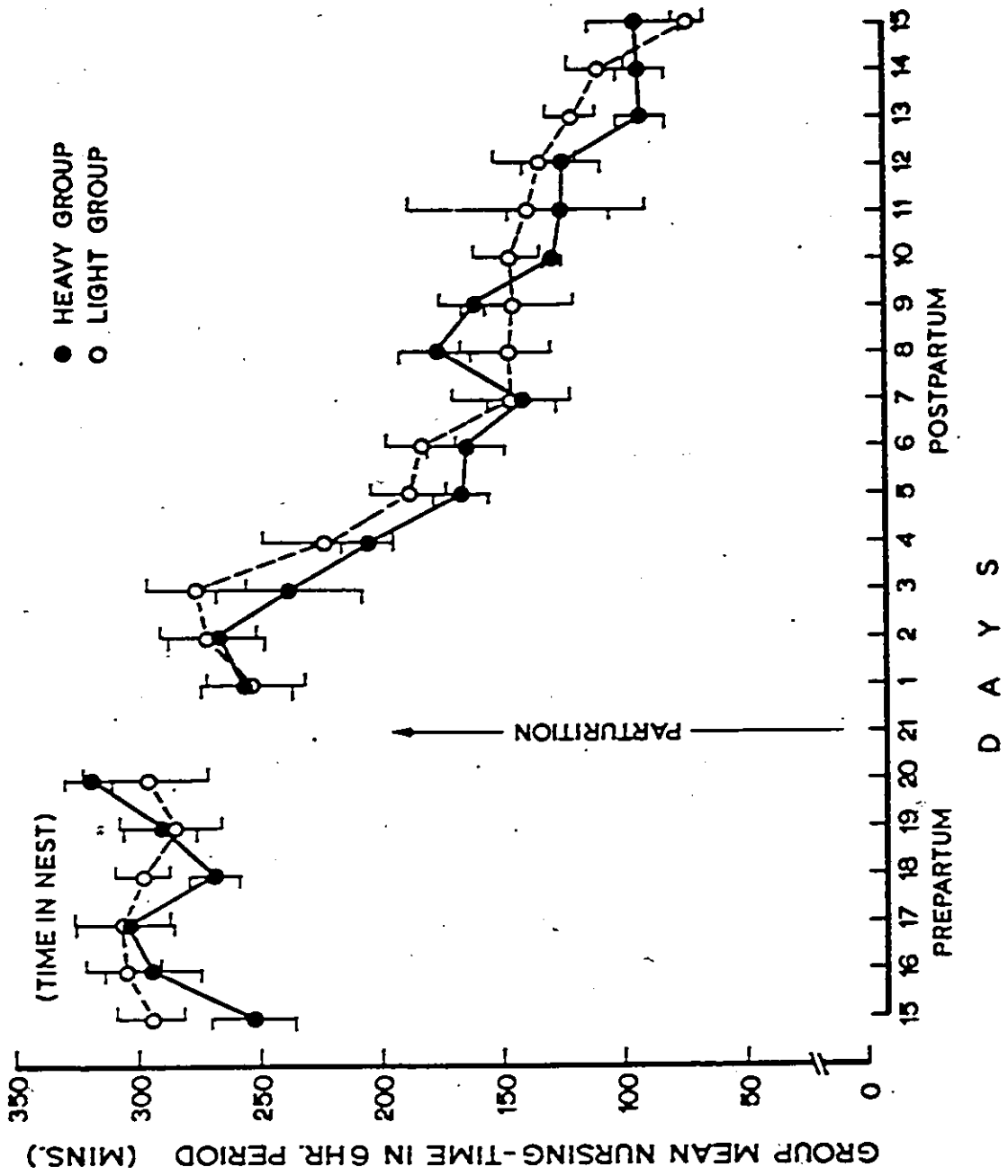
● HEAVY GROUP
○ LIGHT GROUP
PARTURITION
PREPARTUM
POSTPARTUM
DAYS
(TIME IN NEST)
GROUP MEAN NURSING-TIME IN 6HR. PERIOD (MINS.)
350
300
250
200
150
100
50
0

Figure 16

Figure 17

Experiment V(b): nursing frequency through the postpartum period (based on 6-hour daily observation period).

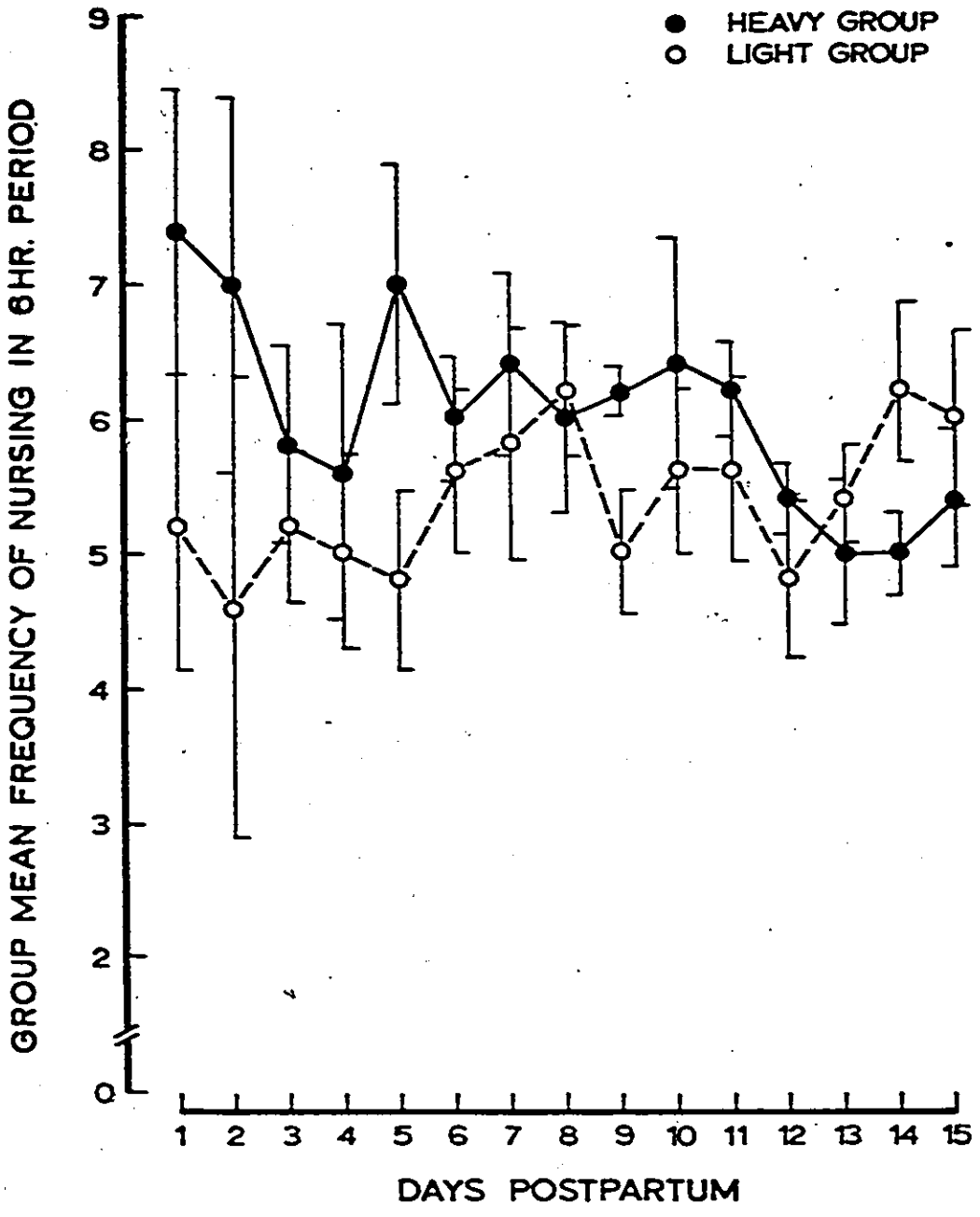
HEAVY GROUP
LIGHT GROUP
GROUP MEAN FREQUENCY OF NURSING IN 8HR. PERIOD
9
8
7
6
5
4
3
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS POSTPARTUM

Figure 17

Experiment VI

DESIGN OF A CONTINUOUS RECORDING SYSTEM FOR NEST-TIME OF THE LACTATING RAT

Nest-time, i.e., the time when the mother is actually on the nest, is easily distinguishable when the rat is in the standard laboratory cage. Here, the mother usually constructs a nest by piling sawdust up into a mound in one of the back corners of the cage. The litter either rests in a central depression in the mound, or between the back and side walls with the mound forming the third side of a triangle. The mother usually enters the nest carefully, and takes less than a minute to settle into the nursing position over the litter. She will usually leave the nest with similar care dragging her ventral surface over the edge of the mound. This serves to ease the pups off the nipples and prevents them from becoming isolated from the nest. Thus, the ON-nest and OFF-nest states are normally quite discrete. However, sudden disturbance or interference by the experimenter frequently results in the mother leaving the nest quickly and dragging pups with her still attached to her nipples. Pups that become isolated under these conditions are usually soon retrieved but occasionally pups partly covered by sawdust may remain isolated for some considerable time. On other occasions mothers will simultaneously maintain two or three smaller nests with

the litter thus split into several parts.

In order to monitor continuously the amount of time a mother spent with her litter, it was initially decided that the normal laboratory conditions should be preserved as far as possible. To this end, attempts were made to record entries and exits from the sawdust-mound nest using different devices (proximity-detector plates, photo-electric cells, tilting platforms, etc.) none of which proved satisfactory. However, a system was eventually developed which involved instead providing the mother with an already prepared nest to which there was no reasonable alternative. Although some residual problems remain with this system, a full record of nest-time throughout the first two postpartum weeks has been achieved for the majority of mothers studied.

Design of recording system:

The standard laboratory clear plastic cage (34 x 29 x 17 cm) was fitted with a wooden partition (A in Figure 18a) 12 cm from one of the short sides. A rocking tray (B) containing sawdust was placed behind the partition. The tray could be balanced with metal washers so that it tilted when the mother stepped onto the nest and returned when she left it. A microswitch mounted on the partition was closed while the mother was on the nest and open when the litter was unattended. To encourage the mother to nest in the tray, a 1 cm square mesh wire grid (D) was placed 2 cms above the floor of the cage's outer compartment. This provided an unattractive alternative to the prescribed nest site, and an area for absence from the nest, feeding, drinking, urination, and defecation. Nest material was provided only in the rocking tray which was shielded from light by an L-shaped metal plate (C) fixed to the food hopper. This plate also prevented the mother from feeding while on the nest.

The rocking nest-tray was constructed of sheet aluminium formed on an inverted T-shaped plywood frame (see Figure 18b). At the entrance to the tray, the metal was bent to form a lip which prevented debris from falling out of the nest and hindering the operation of the recorder. A stainless steel bar fixed to the bottom of the tray formed a fulcrum which engaged with two notched plastic retainers glued to the cage bottom. Two $\frac{1}{4}$ x 2" bolts were fixed to the frame on the side opposite the tray to

hold balancing washers. The rocking tray and cage partition were finished with non-toxic, flat black paint.

Since the microswtich is closed as long as the mother is on the nest, the data may be recorded on moving chart paper which provides a complete history of the nesting behaviour, or may be reduced in a number of ways. Figure 19 shows a circuit that was used in Experiment VII for obtaining total nesting time and the number of nesting episodes as well as the chart record of the nesting pattern. The mother rat frequently steps briefly into the nest and leaves again without nursing. She also tilts the nest with her tail or stands briefly with her forepaws on the edge of the nest without entering. The circuit simply delays a relay closure for a preset period of time and thereby eliminates such spurious brief microswitch closures. It was found that a 5 second delay eliminated most of these unwanted actuations of the microswitch yet preserved all nesting episodes in which the pups were given an opportunity to be nursed. The component values given in the figure will provide a 5 second delay.

Figure 20 shows two sample records from mothers on a 12 hour light (0800 - 2000 hrs) dark (2000 - 0800 hrs) cycle for the last few days before parturition and for the first 15 postnatal days. Nest time is not shown on prenatal Day 21 as the cages were disconnected several times for observations of parturition. Both mothers reared litters culled to nine pups on postnatal Day 1. In both records there was a

distinct difference between the total nest times for the light and dark periods which suggests that the mother's nesting-behaviour is affected differently by the light and dark cycle. Invariably the mother spent less time in the nest in the dark than she did in the light. For both mothers the dark-light difference was quite marked in the last few days of gestation. Over two thirds of the total time was spent in the nest during this period. In the postpartum period both mothers showed a progressive decline in nest time, until by the end of the second week the total nest time for a complete day was less than six hours.

Considerable differences were apparent between mothers. In the upper record of Figure 20 the circadian pattern was maintained for almost two weeks postpartum, whereas in the lower record the pattern disappeared by about the ninth day. The latter case was representative of the lower limit of loss of the circadian rhythm that has been observed; in several of the mothers studied a definite rhythm persisted as far as the fifteenth day when recording was stopped. At about this time, the pups begin to make excursions from the nest and may initiate nursing in the outer compartment.

On this occasion, and in subsequent experiments in which the recording cage was used, all mothers gave birth in the nest tray and only one stopped using it as the nest for the two week postpartum period. Data to be presented in Experiment VII and VIII will show that in the majority of cases parturition occurs in the light period and

the time of birth can therefore be obtained by direct observation. In some cases, however, mothers do give birth in the dark period and a good approximation of the time can be obtained from the event record as there is a marked cessation of activity 30-90 mins prior to delivery. Further observations of the state of the litter (e.g., how clean the pups are; whether milk is visible through the abdominal wall or not; the presence or absence of placental material) may be used to substantiate the estimate of birth time.

As well as providing a continuous record of maternal nest time, the system also sets up unusually stable nesting conditions. In the description of nursing in the standard cage, given earlier, it was noted that pups would occasionally become isolated from the nest, and that infrequently the mother divides the nest into several smaller parts. In the recording cage a pup will occasionally slip out of the nest tray but is usually retrieved without the mother having to leave the nest. The sightless pup will often climb back over the lip into the nest, presumably in response to acoustic, thermal, or olfactory cues. The lip at the exit of the nest tray provides a small cliff which, although often inspected by the pups, prevents them from going any further. The nest in the recording cage therefore minimises the likelihood of isolation and, as qualitative differences (number and type of nest) due to the mother are not possible, the recording nest remains as the single nursing unit. All litters are therefore subjected to a more uniform experience in the first

two critical weeks of life, and this appears to substantially improve the quality of observations on development. Periodically, litters reared in these recording cages have been compared with those reared in the standard cage in which sawdust alone was provided for nesting. Fewer instances of runts and less within-litter variability in growth rate and development have been recorded in litters reared in the recording cage. The impression gained from these observations is that the constancy of conditions in the nest site in the recording cage may facilitate maternal care. Also, experimenter interventions seem to be less disruptive in the recording cage.

Obviously it is important to know how closely nest-time approximates nursing time. The compactness of the nest facilitates orientation towards the nipple by the pups when the mother enters the nest and, as she leaves the nest, she drags her ventral surface over the lip of the nest-box which prevents any of the litter from falling out of the nest. There seems to be insufficient room for the mother to be on the nest and not nursing in this apparatus, and we have invariably found that pups are suckling when the mother is lifted off the nest. The mother can neither feed nor drink when she is with the litter and, while she may groom some pups on first entering the nest, other pups are suckling within seconds. Furthermore, observations of mothers in a glass-bottomed nest showed that the mother was being suckled practically the whole time that she was on the nest (Gustaffson, 1948). For these

reasons, it seems safe to assume that the amount of time that the mother is observed to be on the nest in this system is a fairly reliable estimate of true nursing time.

Comparison with other studies

At about the time that these preliminary results were obtained with the recording cage, we became aware of a study by Grota and Ader (1969) in which, using a continuous recording procedure, similar results had been obtained, i.e., dark-light differences in nest time, and a progressive decline in nest time over the first two postpartum weeks. The results of both the present study and those of Grota and Ader (1969) are inconsistent with the observations of others on changes in nursing time in the postpartum period (Gustaffson, 1948; Rosenblatt and Lehrman, 1963; Moltz and Robbins, 1965; Holland, 1965; Rosenblatt, 1969). With the exception of the Gustaffson (1948) and Holland (1965) studies, the results above were based on short observation periods in the light. period. The results obtained here and by Grota and Ader (1969) suggest that such sampling was not representative of the changes occurring in nursing behaviour in the postpartum period. The data of Rosenblatt (1969) showed little change in the percentage of mothers observed to be in the nest over the period 1-12 days, while the results obtained by Moltz and Robbins (1965) showed a significant increase in nursing time from postpartum Day 2 to the end of the third week. The results obtained in Experiment Va (Figure 14) which were derived from three hours of

daily observation also showed no decline in nursing time over the first three weeks.

However, data based on short periods of sampling are not always unrepresentative. In a later study by Moltz, Geller, and Levin (1967) control mothers showed a progressive decline in nursing-time over the first three weeks, and the results obtained in Experiment Vb clearly show a decline in nursing time over the first two weeks. Also a study by Deitchman (1970), in which three 12-minute samples were taken daily, showed both a decline in nesting behaviour over the first three weeks, and dark-light differences. Two of the sampling periods were made in the early part of the light period and the third was in the dark period using low-level red illumination.

Although the continuous recording procedure is a first step in resolving many of the problems that have arisen in studies of maternal behaviour, the several continuous recording studies, used this far, have not yielded a consistent assessment of the quantity of change in nursing behaviour that occurs in the postpartum period. The data obtained by Gustaffson (1948) show no substantial change in nest-time over Days 4-9 and, surprisingly, the mother spent more time in the nest in the dark period than in the light period. Continuous recording was achieved by placing a movable weight-sensitive disc underneath the nest. In a study by Holland (1965) the standard cage was pivoted so that when the mother moved into the nest compartment her weight tilted the cage

which then operated an elapsed time meter. The results[1] revealed only a 20% reduction in the total time available for nursing over the first two weeks. However, the dimensions and shape (6" x 10") of the nest compartment would have allowed the mother ample room to remain in the nest compartment and not be nursing. This, and other factors, e.g., strain differences, differences in litter size (see Grota and Ader, 1969) may have accounted for the exceptionally high values obtained. Data that have been obtained using the system described here are generally comparable with those of Grota and Ader (1969). Their salient features and findings are compared in the table below:

Table XXI

	Nest area (sq. ins)	Size of Litter	Percent of total time spent with the litter		
			Day 2	Day 8	Day 15
Dual-chambered cage (Grota and Ader, 1969)	81	8	80	62	38
Single cage (present work)[2]	35	8	67	33	17

[1]Based on the estimated mean from the Maudsley reactive and non-reactive strains that were studied.

[2]Based on six control mothers from Experiment VII.

Grota and Ader (1969) used a dual-chambered system consisting of two identical square plastic cages (9 x 9 x 9 ins) connected by a cylindrical passageway through which the mother could pass. Food and water were available in both cages. It can be seen in the table above that although both sets of mothers nursed the same size litter, the total time spent with the litter was consistently lower in the present study. These differences may have been due to the area and nature of the respective nest compartments. The nest area in the Grota and Ader (1969) study was considerably larger than that used in the present study and it is possible that the mother was able to spend time in the nest cage and not be nursing, particularly if the nest was concentrated in one of the corners. In addition, their mothers could obtain food and water in the nest cage. In the present system, however, the mother cannot spend time with the litter and not be nursing, and she is also obliged to leave the nest for food and water. It would appear, therefore, that the present system provides a closer estimate of true nursing time, and a better opportunity to detect individual differences.

SUMMARY AND CONCLUSIONS

A recording system is described which was designed to monitor continuously the amount of time that a mother rat spends with her litter. The results obtained with the system support the findings of others that nest-time shows a progressive decline over the first 15 postpartum days (Holland, 1965; Grota and Ader, 1969; Deitchman, 1970) and that mothers spend more time on the nest in the light period than they do in the dark period (Grota and Ader, 1969; Deitchman, 1970).

It appears that by keeping nest area at a minimum, and making food and water available only outside the nest, the recording of nest time provides a closer approximation of nursing time than has been achieved in other continuous recording systems, and therefore increases the probability of detecting individual differences in maternal behaviour.

The properties of the artificial nest used in the present system appear to reduce qualitative variation among mothers, and the constancy of conditions in the nest appears to impart more uniformity to the early experience of litters which may minimise variability and thereby improve the quality of observations on early development.

Figure 18

Exploded view of recording cage to scale (a) and enlarged front view of removable nest-box (b).

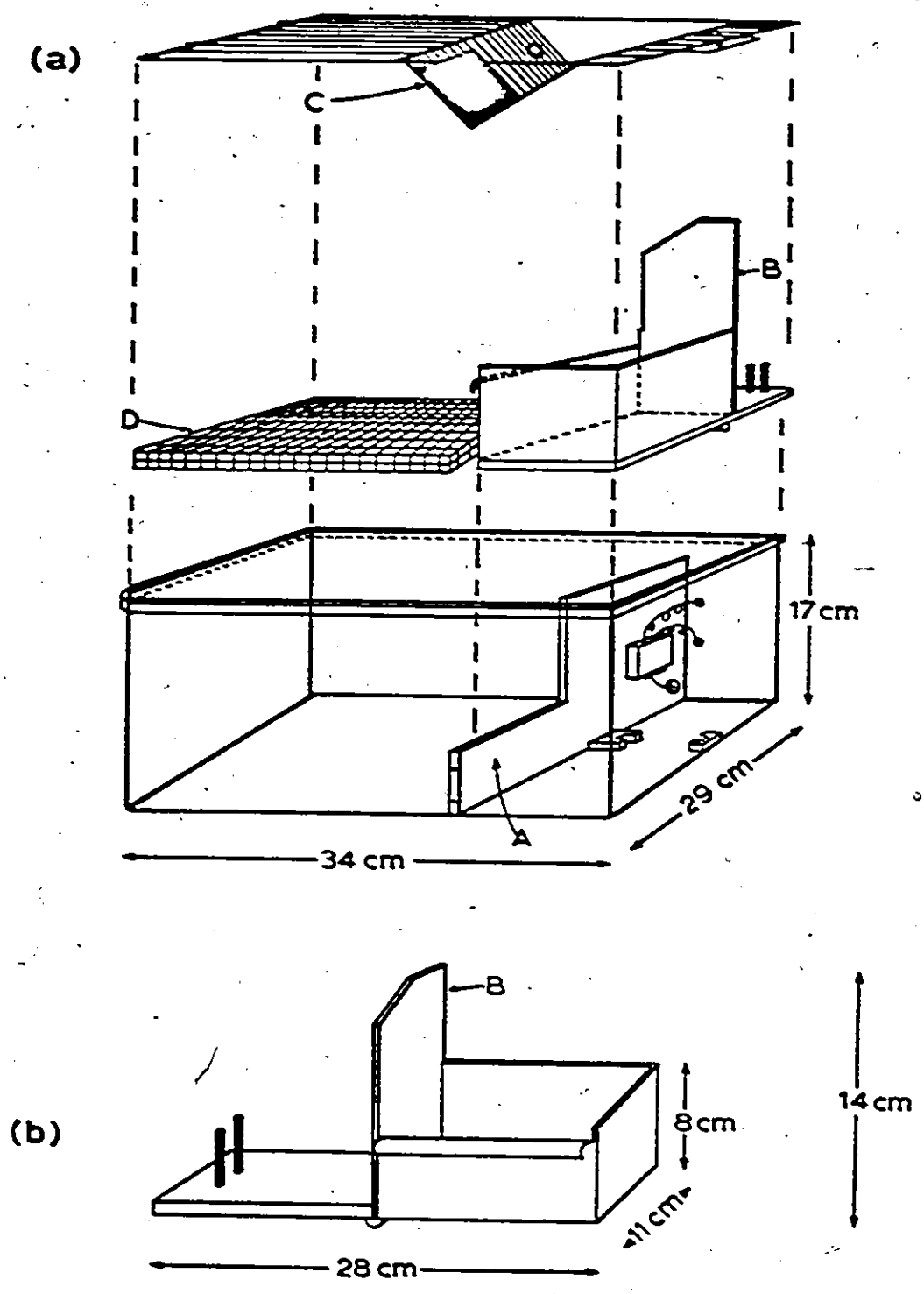

Figure 18

Figure 19

Schematic diagram of time-lag circuit. Resistor values are in ohms, and capacitor values in microfarads. Ry is a Potter and Brumfield 24V relay, KA 11 DY.

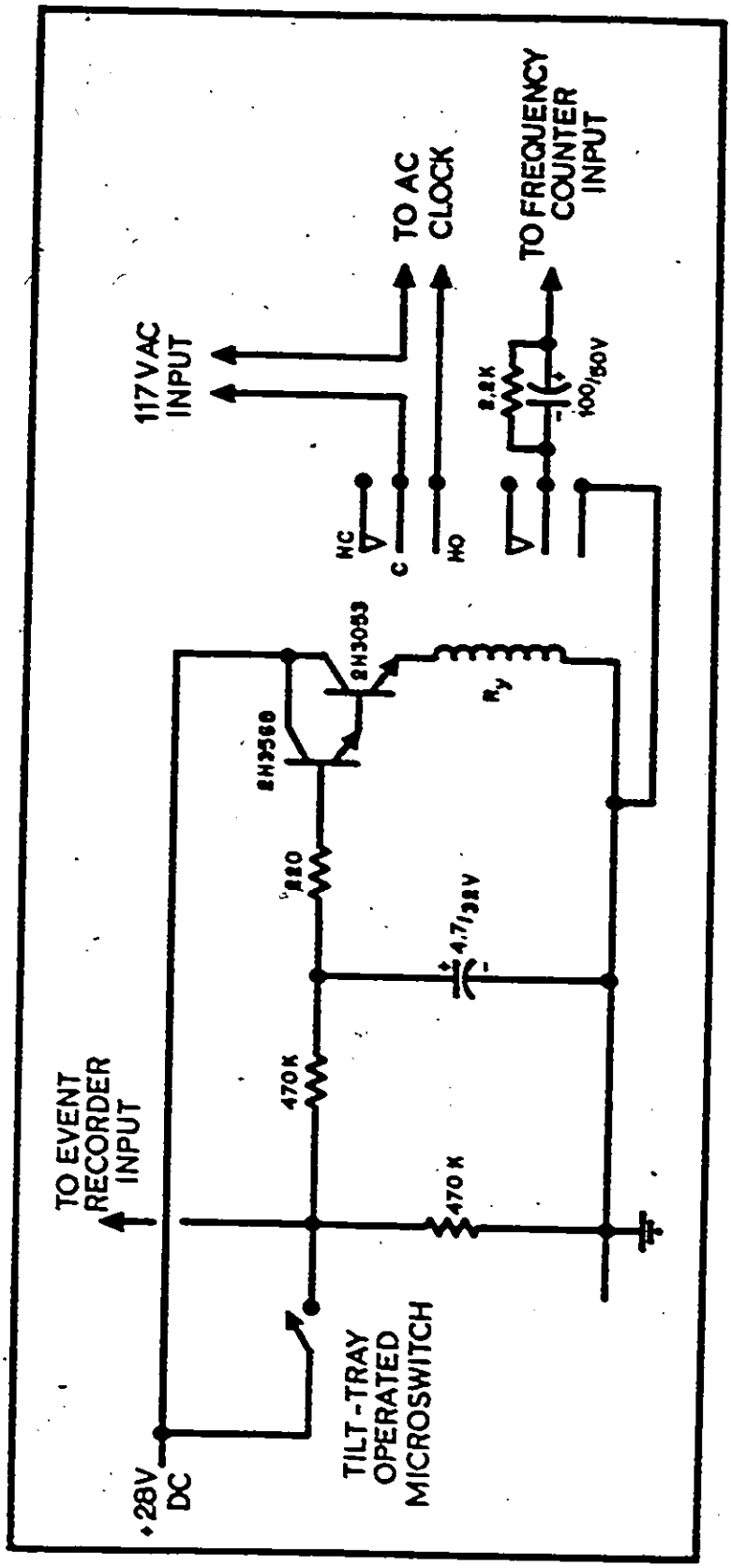
117 VAC INPUT
TO AC CLOCK
TO FREQUENCY COUNTER INPUT
NC
C
NO
2.2K
100/50V
2N3053
2N3568
220
4.7/25V
470K
470K
TO EVENT RECORDER INPUT
+28V DC
TILT-TRAY OPERATED MICROSWITCH

Figure 19

Figure 20

Sample records from two individual mothers showing total time spent in the nest in the light and dark periods in late gestation, and in the first 15 postpartum days.

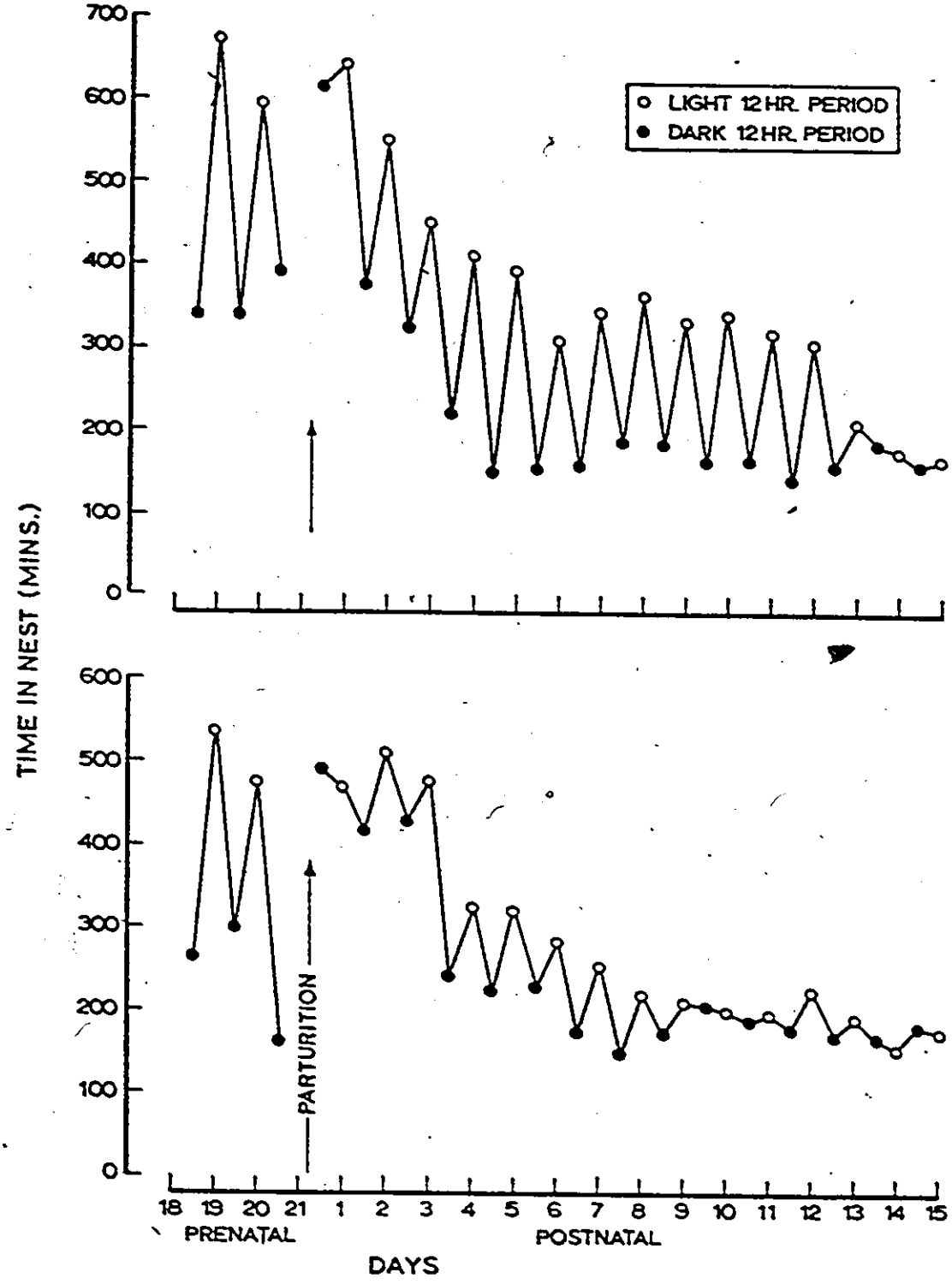
LIGHT 12 HR. PERIOD
DARK 12 HR. PERIOD
TIME IN NEST (MINS.)
700
600
500
400
300
200
100
0
PARTURITION
18 19 20 21 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
PRENATAL
POSTNATAL
DAYS

Figure 20

Experiment VII

INTERRELATIONSHIPS BETWEEN MATERNAL BODY-WEIGHT, NURSING BEHAVIOUR, AND DEVELOPMENT AND ADULT BEHAVIOUR OF OFFSPRING

The results of Experiments Va and Vb indicated that the amount of time spent nursing by the mother in the postpartum period might be related to her body-weight, although no firm conclusions could be drawn on the basis of the data. The continuous recording system (Experiment VI), however, seemed likely to provide more complete data on the amount of time spent by the mother with her litter, as well as the frequency and duration of the nursing periods in the dark and light cycles.

The objective in designing a continuous recording system was to obtain nursing profiles of mothers who had been prenatally treated with growth hormone. There was reason to believe that the increases in maternal body-weight that resulted from the treatment might be associated with differences in nursing behaviour. However, before that study (Experiment VIII) was attempted, the present study was undertaken to assess the influence of maternal body-weight on nursing behaviour, development of the litter, and later behaviour of the offspring in adulthood. The possible influence of maternal body-weight on length of gestation, parturition, and parameters of the litter (size and weight), and the

possible prenatal regulation of maternal lactation potential, were also investigated.

Through the simultaneous investigation of these parameters it was hoped to disentangle the various components of maternal behaviour and, thus, help clarify interpretations of the effects of early experience.

It must be recognized at the outset that a study of maternal body-weight is at the same time a study of the variables which determine it (see p. 188). Under normal conditions of growth in the rat, and particularly in the case of a highly inbred strain such as the one used here, age is the principal determinant of body-weight. In the present study, it may be assumed that the range of body-weight studied was associated primarily with a concomitant age-range and that some small part of the variation was accounted for by genetic, ontogenetic and constitutional factors.

The question of the exact aetiology of these weight differences may ultimately prove important (see pp. 294 - 296; 372 - 375; 382). The present study is a first step in the systematic investigation of the relationship between maternal body-weight and maternal behaviour. In a later study (Experiment VIIIb) in which differences in maternal body-weight of a known aetiology were studied, differences in maternal behaviour were quantitatively similar to those observed here and previously (Experiments Va and Vb)

METHOD

Subjects were primiparous Long-Evans rats from Blue Spruce Farms. Following one week of acclimatisation to the laboratory on a 12-hour light (0800 - 2000 hrs) dark (2000 - 0800 hrs) cycle, subjects were mated and insemination confirmed by vaginal lavage. A sperm-positive test defined Day 0 of pregnancy. If mating was successful, subjects were assigned to one of two groups on the basis of their body-weight on prenatal Day 1. Those in the weight range 195-210 gm. were assigned to the light (L) group and those in the range 220-245 gm. to the (H) group. Additional mothers outside this range were studied as far as parturition. Length of gestation was arbitrarily timed from 0200 hr. on Day 0 of pregnancy.

All subjects were housed in standard clear plastic cages and body-weights were recorded daily around midday. On Day 18 of gestation, subjects were transferred to continuous recording cages (see Figure 18, Experiment VI). Readings of total time in the nest-box and the frequency of nest-periods were taken twice daily at 0800 hr. and at 2000 hr. As soon as possible following parturition, the number and total weight of the litter and maternal body-weight were recorded.

On the day following birth all litters were culled to eight pups comprising four males and four females. In a few cases where the litter had insufficient numbers of either sex, spare pups of the same age and from

the same group were substituted. Cross-fostering was then effected so that four groups were arranged to control for prenatal and postnatal effects (Table XXII). All litters were cross-fostered and sixteen litters (four in each group) were selected for future study. The sixteen mothers and their litters were kept in the recording cages until postnatal Day 15 when they were transferred back to the standard cages. Litters were weaned on postnatal Day 25 and littermates housed, by sex, four to a cage.

In the postnatal period mothers were weighed daily and the cumulative frequency and duration of nursing were recorded twice daily as before. The following observations of morphological and reflex development of the pups were made in the postnatal period.

Body-weight:--On postnatal Days 1 and 5, the collective weight of the four males and the four females were taken separately on a spring balance scale. On Day 10, and at five day intervals up to Day 50, pups were sexed and weighed individually.

Eye opening:--On Day 12 daily observations were begun on each pup to determine the time of eye-opening. Each eye was scored from 0 (no break in the eye-lides) to 3 (fully open); the maximum score for both eyes fully open was therefore 6. This method of scoring permitted an assessment of the degree of eye-opening at a particular age as well as providing a record of the age at which the first opening of an eye occurred.

Startle Reflex:--Commencing Day 10 each pup was tested for its response to a characteristic and repeatable high-pitched noise made by

the experimenter through pursed lips. A "startle" was recorded when the pups, held in the experimenters' hand, made a noticeable sudden response to the sound stimulus. The emergence of the reflex appeared to be all-or-none and the response was scored either -ve (absent) or +ve (present).

Free-Fall Righting Reflex: --Commencing Day 15 pups were tested on their ability to right themselves in mid-air. The pup was held for several seconds in the inverted position at a height of 30 cm. above a cotton wool pad and then released. The age at which the pup landed on all fours was recorded. The response was recorded as -ve (incomplete righting) or +ve (complete).

Vaginal Opening:--Commencing Day 32, females were weighed daily and the vagina was examined. The beginning of vaginal opening was evidenced by the appearance of two lateral folds which, over the next few days, deepened until the dividing septum finally parted. The age at which this complete opening occurred was recorded.

On Day 56 the females were sacrificed and the brains removed for the biochemical assay of DNA and protein. Between Days 77-82 the males were tested daily in the open field, a plan of which (together with the observations that were made) is shown in Figure 21. The field consisted of 2 ft. high plywood sides finished in matt-black, and a

clear acrylic floor. The underside of the floor was painted white and squared off with black lines into sixteen 9 inch squares. The field was illuminated by normal ceiling lighting; the luminance in the centre of the field was approximately 3 foot lamberts.

The subjects were housed by litter in the room in which the open-field testing was conducted. The four litter-mates were individually identified by ear-marks as follows: P (no ear marks); L (left ear mark); R (right ear mark); and LR (both ears marked). The order of testing was determined by ear-mark. On a particular day, all the P subjects were run first followed by the L subjects and so on. This ensured that any possible diurnal differences in open-field behaviour were evenly distributed across all litters.

At the beginning of a trial the subject was removed from the home cage and placed in the square marked S with the subjects' head facing diagonally across the field to the corner opposite to the one behind which the experimenter was sitting. The stop-watch was started as soon as the subject was placed in the field. A small mirror mounted on top of one of the side walls at M enabled the experimenter to observe the subject along the nearside part of the field without moving his position. Each subject was observed for 5 min. At the end of the trial, the subject was returned to the home cage and the number of fecal boluses in the field was counted. The floor was swabbed with an alcohol/water solution and the next trial commenced when the floor

was completely dry. Each subject was observed on five consecutive days.

Analysis of data:--For the analysis of the four prenatal/postnatal combinations shown in Table XII an analysis of variance was first performed to determine an over-all F ratio and an estimate of the population error variance. Dunn's Multiple Comparison Test (1961) was subsequently used for a priori comparisons among the four group means.

In order to eliminate bias due to "litter effect" (see King, 1969) individual scores within a litter were averaged to obtain a mean score for the litter. This litter mean score was treated statistically as a sample of one. Where differences due to sex were apparent the data from the four females and four males in each litter were analysed within their respective groups, the sample size again determined by litter and not by individual.

As the distributions of age of appearance of the startle reflex appeared to be skewed (due perhaps to the discreteness of the daily sampling procedure), the ages were subjected to logarithmic transformation in order to normalise the distributions before statistical analysis.

RESULTS

Gestation and Parturition

The body-weight range on Day 0 of pregnancy of 27 mothers studied as far as parturition was 194-284 gm. Of these, 26 delivered their litters between 1130-1700 hr. on Day 21; the remaining litter was delivered at 1300 hr. on Day 22. Thus, all litters were delivered in the light period.

Maternal body-weight on Day 0 was not significantly correlated with mean body-weight of the offspring ($r = +0.21$, $t = 1.03$, $p > 0.05$), litter-size ($r = +0.21$, $t = 1.1$, $p > 0.05$), or length of gestation ($r = -0.20$, $t = 1.01$, $p > 0.05$). The regression of birth-weight (Y) on litter-size (X) was negative ($Y = 5.8427 - 0.191X$) but not significant (p (zero slope error) > 0.05). The regression of gestation length (Y) on litter-size (X) was negative ($Y = 523.606 - 0.616X$) and significant ($p < 0.01$). The calculated values of gestation length for the different litter-sizes are given below (the litter born on Day 22 is excluded from the analysis.

Table XXIII

Calculated values of gestation length for litter-size

Number of Litters	Litter-size	Gestation Length (hours)
2	15	514.365
3	14	514.981
7	13	515.597
6	12	516.213
6	11	516.829
2	10	517.445

Maternal Body-weight

On Day 0 of pregnancy the eight heavy mothers were on average 33 gm. heavier than the eight light mothers. Both groups gained approximately the same amount of weight during the course of pregnancy, and at Day 21 the average difference between the two groups was 33 gm. (Figure 22). The postnatal weight curves are very similar for both groups. In the first 24 hr. following parturition body-weight dropped slightly. Over the next 12 days there was a steady increase followed by a plateau and then a decline towards weaning (Day 25). With the pups removed there resulted an initial increase, followed by a further decline to a trough between Days 27-32; thereafter, there was a slight increase up to Day 40 when observations ceased. Throughout the prenatal and postnatal periods the body-weight difference between the two groups on any day was statistically significant (p (at least) < 0.05).

Following parturition, the light group of mothers retained slightly

more weight than the heavy group, and over the next 40 days the average difference between the two groups was progressively reduced, mainly in the period between the plateau (Days 12-14) and weaning. On Day 40 there remained a significant difference ($p < 0.05$) of 18 gm. between the two groups. The net increase in body-weight from the start of pregnancy to the postweaning plateau was significantly greater ($p < 0.05$) in the light group (63.38 ± 5.7 gm.) than in the heavy group (44.75 ± 4.4 gm.)

The 16 mothers assigned to the four prenatal/postnatal conditions (see Table XXII) were compared for their mean body-weight over the first 15 postnatal days in the same way that their litters were later compared.[1] The hypothesis:

$$H_1 : \tfrac{1}{2}(H/H + H/L) \neq \tfrac{1}{2}(L/H + L/L)$$

was accepted ($p < 0.05$), while

$$H_1 : \tfrac{1}{2}(H/H + L/H) \neq \tfrac{1}{2}(H/L + L/L)$$

was rejected ($p \geq 0.05$) i. e., the two postnatal heavy mother groups (H/H and H/L) were significantly heavier than the two postnatal light mother groups (L/H and L/L), while the two groups rearing pups from heavy mothers (H/H and L/H) were not significantly different from those rearing pups from light mothers (H/L and L/L).

---

[1] Dunn's Multiple Comparison Procedure (in Kirk, 1968).

Prenatal : Postnatal Litter Ratio

Similar comparisons were made among the four groups for their respective prenatal:postnatal litter-size ratios, i.e., the ratio between the number of pups born to a mother and the number she subsequently reared (which in all cases was 8). The analysis resulted in acceptance of the hypotheses:

$$H_o : \tfrac{1}{2}(H/H + H/L) = \tfrac{1}{2}(L/H + L/L)$$

$$H_o : \tfrac{1}{2}(H/H + L/H) = \tfrac{1}{2}(H/L + L/L)$$

i.e., that prenatal:postnatal litter-size ratios were not significantly different between the two postnatal heavy mother groups (H/H and H/L) and the postnatal light groups (L/H and L/L), nor between the two groups rearing offspring from heavy mothers (H/H and L/H) and those rearing offspring from light mothers (H/L and L/L).

Maternal Behaviour

(1) Nest-time:--Total time spent in the nest-box and the frequency of nesting periods was recorded for five heavy and six light mothers. Records from the remaining five mothers were incomplete due to intermittent apparatus failure and are not included in the analysis. The statistical analysis of the data is based upon the 14 complete days during which a litter was with its foster mother. The period following parturition until fostering was effected on Day 1 is not included as during this time the mother was with her own unculled litter; neither is the dark period of Day 1 included as this would include a half-day

in the analysis. The data are summarised in Table XXIV.

In the late prenatal period and throughout the postnatal period, the circadian pattern of nest-time is apparent in both groups. On prenatal days 19 and 20 the amount of time spent in the nest in the light period is roughly double that in the dark period. On postnatal Day 2 the ratio is more than double, but through the postnatal period it progressively decreases as the circadian difference is gradually lost.

On prenatal Days 19 and 20 the heavy group spent less time in the nest in both the light and dark periods compared with the light group. In the postnatal period (Fig. 23) both groups showed a decline in nest-time over Days 2-15; on postnatal Day 2, the total time spent in the nest constituted about 50-60% of the total time available whereas by Day 15 it was less than 20%. The rate of decline does not appear to be uniform through this period; in the heavy group, there is a slight "bump" between Days 7-9, and a similar discontinuity appears in the curve of light group after Day 10. Throughout these 14 postpartum days, values for the heavy group fell consistently below those for the light group. Total average nest-time was 5469 $\pm$ 440 min. for the heavy group which is significantly less than that for the light group, 6788 $\pm$ 256 min. ($p < 0.05$). Thus, over the 14 days the light-group mothers spent 25% more time with their litters than did the heavy-group mothers.

For the eleven mothers, a significant negative correlation was found between maternal body-weight on Day 0 of pregnancy and total

nest-time over Days 2-15 ($r = -0.70$, $p < 0.01$). As maternal body-weight in the postnatal period (Days 1-15) is largely determined by body-weight at the start of pregnancy a negative correlation was also found between postnatal mean maternal body-weight (over Days 1-15) and total nest-time over this period ($r = -0.69$, $p < 0.02$). Nest-time was not significantly correlated with the prenatal:postnatal litter size ratio ($r = -0.02$, $p \geq 0.05$).

(2) Frequency of nest-periods:--The paper chart event record was analysed to provide information on the frequency characteristics of nest periods. Any interval of less than one minute on the nest was excluded from the analysis. The frequency of nest-periods in excess of one minute was recorded for both dark and light periods over postnatal Days 2-15 and are summarised in Table XXV.

In late gestation, both groups showed a lower frequency of nest-periods in the light period than in the dark period. In the postnatal period, the frequency in both the light and dark periods showed a slight decline in both groups over the 14 days although the rate of decline was not uniform (Figure 24). Total daily frequency for the two groups is shown in Figure 25 and indicates a noticeable decline in the first week, followed by a slight elevation in the middle period, and a second decline over the last four days. Overall, values at the end of the second week were lower than those in the early postnatal period.

In the first postnatal week the frequency of nest-periods in the

light period tended to be higher for both groups than in the dark period whereas in the second week the reverse appeared to be the case. This results in the total frequency for the two periods being virtually identical within groups (Table XXV). Mean daily frequency for the light group was greater than that for the heavy group on 12 of the 14 days but the difference between the total mean frequency of the two groups is not statistically significant ($p > 0.1$).

In order to obtain information concerning the duration of individual nest-periods, each discrete nest-period was allocated to a 5-minute temporal bin according to its duration. This procedure was followed for each animal on prenatal Days 19 and 20 and postnatal Days 2-15. The group mean data for light and dark periods for the light group are presented in Figure 26 and for the heavy group in Figure 27 on four representative days through the period studied. The ordinate values are obtained by expressing the mean frequency of a particular bin as a percentage of the total frequency of nest-periods in a particular light or dark period.

For both groups, the frequency distribution of nest-periods appears to be more truncated in the dark period compared with the light period on any given day, i.e., the distribution is positively skewed with nest-periods of short duration occurring more frequently. This difference is evident before parturition. During the light period on prenatal Day 20, the relative frequency of nest-periods spans the full range of bins,

some of the periods being in-excess of 150 min. In the dark period of the same day, the longest nest-period in either group was never in excess of 80 min. The distributions for both light and dark periods are skewed towards the higher frequencies which are represented in the nest-periods of short duration; the modal frequency always falls in one of the three shortest bins (1-5, 6-10, 11-15 min.).

The frequency distributions of light and dark periods show a contraction through the postpartum period. A progressive diminuition occurs in the duration of nest-periods until, by the end of the second week, periods in excess of 45 min. did not occur. The tendency by both groups to show this contraction in frequency range, which is accompanied by only a slight drop in total daily frequency, resulted in a shortening of the average nest-period through the postpartum period. This is demonstrated in Figure 28 in which the group daily nest-time is divided by the group daily frequency.

No consistent differences are seen between the frequency distributions of light and heavy groups other than a tendency for the modal frequency to be in a bin of shorter time duration in the light group. This difference appears to hold throughout most of the postpartum period: in the light period, modal frequency was shorter in the light group on nine out of eleven days with three days equal, and, in the dark period, shorter on five out of six days with eight days equal (Table XXVI).

The frequency distributions of off-nest periods on postnatal days 2, 8, and 14 are shown in Figure 29 for the light group, and in Figure 30 for the heavy group.

Pup body-weight:--As all of the litters studied were of equal number and contained equal numbers of either sex, the analysis of pup body weight in the postpartum period can be simplified by considering the whole litter weight through the period when the mother is the only source of nutrition. Mean litter weights in each of the two groups of mothers on Days 1, 5, 10, 15, and 20 are shown in Figure 31. By Day 15, the two heavy-reared groups of litters were heavier than the two light-reared groups. A nested-factors analysis of variance of individual pup weights at 15 days yielded significant ($p < 0.01$) main effects due to postnatal mother, sex, litter, and prenatal mother; the interaction prenatal mother x postnatal mother was also significant (Appendix B). In support of the postnatal mother effect, mean body-weight of the foster mother over Days 1-15 was significantly correlated with pup body-weight at 15 days ($r = 0.57$, $p < 0.02$).

As no significant correlation had been demonstrated between prenatal maternal body-weight and birth-weight of the pups, the significant prenatal effect, and the significant prenatal x postnatal interaction suggest that some feature other than prenatal body-weight of the natural mother was involved, although it is possible that the first day of nursing by the natural mother (prior to fostering) may have been

responsible for the effect. The only other source of variation apparent, aside from genetic differences, is the prenatal litter-size of the foster mother. That this is involved is suggested by the finding that litter weight at Day 15 was significantly correlated with prenatal litter-size of the foster mother ($r = 0.49$, $p < 0.05$). The multiple correlation of these two predictor variables of pup body-weight, i.e., maternal body-weight in the postpartum period, and prenatal litter-size of the foster mother, was 0.65, and these two factors therefore account for over 40% of the variance in pup weight at this time.

Inspection of the data suggested that the effects of these two variables may not be simply additive. In order to eliminate the slight disparity in litter-weights that existed on Day 1 prior to fostering, the percentage gain in litter-weight by Day 15 was compared with foster mother body-weight over the range of prenatal : postnatal litter ratios that occurred in the study. Figure 32 shows the calculated regression lines for percent increase in litter-weight against maternal body-weight for each of these ratios. It appears that for a low ratio (10:8) maternal body-weight is a good predictor of pup weight increase. As the ratio increases, however, the slope of the regression line drops such that when the ratio is high (14:8) a mean difference of over 60 gm. in maternal body-weight has little influence on pup weight increase.

The difference in litter-weight between the heavy-reared (H/H and H/L) and light-reared (L/H and L/L) groups continued to widen

through the preweaning period. By day 25, the mean difference between the groups was 27 gm. although considerably more variance was apparent as the pups assumed feeding autonomy. In the postweaning period differences between groups decreased, and, by the time of autopsy of the females at 56 days body-weight equality was observed between the groups (Table XXVII). Mean body-weight of the males at 90 days was also comparable between groups (Table XXVIII).

Eye-opening:--No eye-opening was observed in any pup before Day 14 and both eyes were fully opened in all pups by Day 17. No differences in the age at which the first opening occurred, or in the degree of eye-opening at a particular age, were apparent between the groups. Neither were there any ostensible differences due to sex of pup (Figure 33).

Startle reflex:--The startle reflex was not detected in any pup before Day 11 and was present in all pups by Day 12. The age at which the reflex appeared in each pup was log-transformed and litter-means in the four groups were compared. Pups reared by the heavy mothers (H/H and H/L) showed a significantly earlier appearance of the reflex ($p < 0.05$) compared with those reared by light mothers (L/L and L/H). A comparison on the basis of prenatal origin of the pups (H/H and L/H against H/L and L/L) revealed no significant difference. In all groups, significantly (p 0.05) more females exhibited the reflex at 11 days than did males (Figure 33).

Free-Fall Righting Reflex:--The first successful completion of this reflex was observed at 17 days and all pups demonstrated the reflex by 22 days. The distribution of age of appearance of the reflex was more nearly normal and comparisons between groups were made on the mean age of appearance of the reflex for the litter. Heavy-reared litters showed a significantly earlier appearance of the reflex ($p < 0.01$). The mean age of appearance for groups H/H and H/L was 18.39 days against 19.27 days for the groups L/L and L/H. A comparison on the basis of prenatal origin resulted in no significant difference. Again, females in all groups tended to show an earlier appearance of the reflex (Figure 33).

Vaginal opening:--More variation was observed in the age at which the vagina opened. The earliest that it occurred was at 33 days and in some animals it did not occur until 48 days. Heavy-reared females tended to show later vaginal opening (mean age: 38.0 days) compared with the light-reared females (35.9 days) although these differences were not statistically significant.

Female brain assay:--The results of the female brain assay at 56 days are summarised in Table XXVII. Multiple comparisons between the various group means did not reveal any statistically significant effects due to postnatal or prenatal mother. It is worthy of note, however, that besides complete "catch-up" in body-weight, the parameters of the brain were generally greater in the light-reared groups

(L/H and L/L) compared with the heavy-reared (H/H and H/L) groups. Furthermore, a comparison of these data with the preweaning weight increases indicates a reverse relationship, i.e., whereas on Day 15 the order of litter-weight from the heaviest to the lightest was H/L > H/H > L/H > L/L, the order from the greatest to smallest in terms of brain protein content at 56 days was L/L > L/H > H/L > H/H, and a similar reversal of trend was apparent for brain-weight and brain DNA. A correlational analysis of these brain parameters with pup weight at 15 days generally supported this reverse trend yielding negative correlations (brain-weight: $r = -0.08$; brain protein: $r = -0.5$; brain DNA: $r = -0.42$), although only the correlation between brain protein and 15 day weight is significant ($p < 0.05$).

Open-Field:--A summary of the behavioural parameters (see Fig. 21) of the males in the open-field is provided in Table XXVIII; the data were obtained by averaging the scores for the litters within the four groups across the five days of testing. In general, heavy-reared males tended to defecate more and be less active compared with the light-reared males (Figure 34). The amount of open-rearing tended to increase across the five days of testing whereas close-rearing remained at a fairly constant level. Multiple comparisons among the groups for these parameters of open-field behaviour did not yield significant differences, however, for the eleven litters for which total nursing time data was available, a significant negative correlation was found with defecation

($r = -0.68$, $p < 0.05$) and a significant positive correlation with activity ($r = 0.87$, $p < 0.002$).

DISCUSSION

The finding in the present study that all births occurred in the light phase of the light-dark cycle suggests that parturition is dependent on the photoperiod and is in agreement with other findings on the rat (Blandau and Soderwall, 1941; Mitchell and Yochim, 1970; Plaut, Grota, Ader, and Graham, 1970). An alternative explanation of the tendency to deliver in the light period is that the interval between mating or implantation and delivery is relatively fixed and that parturition only occurs in the light because progestational events (or implantation) are time-locked to the photoperiod (see Porter, 1972). Against this interpretation is the finding that reversal of the cycle on Day 1 of pregnancy results in a backwards or forwards shift of 12 hr. in the distribution of deliveries so that they still occur in the light phase (Plaut, Grota, Ader, and Graham, 1970). Mitchell and Yochim (1970) found that groups of animals exposed to 2, 14, or 22 hours of light daily from the start of pregnancy exhibited a tendency to deliver in the light phase. The possibility exists in both these studies, however, that the time of implantation might have been affected by the changes in the light-dark cycle initiated in early pregnancy. In the Mitchell and Yochim (1970) study, an increasing proportion of light in the cycle was associated with prolongation of pregnancy, although the birth-weights of

litters born on Days 23-24 of gestation were not significantly different from those born on Days 21-22. If no delay had occurred in the time of implantation it would be expected that as gestation was increasingly prolonged birth-weight would increase accordingly.

In the present study, as in the one which follows (Experiment VIII), it was found that birth-weight of litters delivered just before, or in, the light phase of Day 22 were significantly heavier than those delivered in the light phase of Day 21. This indicates that the extension of gestation period in 22-Day deliveries is not due to a delay in implantation, and therefore that parturition is not retroactively timed as a given interval from earlier events in gestation which may be dependent on photoperiod. Rather, it appears that some influence emanating from either the fetuses or the placentae determines when, approximately, parturition will occur (Biggers, Curnow, Finn, and McLaren, 1963); if it does not occur in the prime delivery photoperiod (21 days) then the mother will hold off giving birth until the next photoperiod. As Liggins (1972) notes, it is probably the fetus which determines the day of delivery (say to within 24 hours) but the mother determines the time of delivery (according to photoperiod).

Although Kim, Runge, Wells, and Lazarow (1960) found no evidence of a correlation between litter-size and gestation period, others have reported that large litters tend to have a shorter gestation period than small litters (Mitchell and Yochim, 1970; Smart, Adlard, and Dobbing,

1972). The present data provide a very compelling demonstration of this relationship in that it was observed over a period of hours and not days as in the two studies above. Given the overwhelming tendency for mothers to deliver in the light phase of Day 21, the time of parturition was determined by the number in the litter; large litters were born just after midday while litters in the smaller range were born in the late afternoon.

Previous reports have suggested that birth-weight of the young is influenced by maternal body-weight (Slonaker, 1912; King, 1915; Cole, 1937; Cole and Hart, 1938; Blandau and Money, 1943; Hultquist, 1950; Bateman, 1954; see also Angervall, 1959). In the present study, no evidence for such a relationship was found; neither was maternal body-weight related to litter-size, or to length of gestation period. The mean body-weight difference between the light and the heavy groups at the start of pregnancy was identical to that on Day 21 prior to parturition. As there was no significant difference in mean litter-size between the two groups, the absolute weight of the products of conception must have been comparable in the two groups. These data support the findings described in Experiments III and IV of the present study, and those of Pritchard and Tucker (1970) who noted that improved diet and genetic selection may have resulted in mothers giving birth to heavier litters. Also, improvements in the care and nutrition of laboratory animals, instituted since these earlier studies were done, have probably mitigated

any maternal effects on birth-weight of the offspring.

The relationship between birth-weight and litter-size that others have reported (King, 1915; Benson and Morris, 1971; Smart, Adlard, and Dobbing, 1972) was not investigated as pups were not sexed until postnatal Day 1 and no allowance could therefore be made for the sex-ratio in a litter on the day of birth.

The characteristic change in maternal body-weight in the postpartum period observed in the present study is in close agreement with the data of Ota and Yokoyama (1967a). In the first two postpartum weeks, when the mother is the only source of nutrition for the pups, her body-weight shows a steady increase and this is accompanied by an increase in food-intake (Cole and Hart, 1938; Ota and Yokoyama, 1967a; Altman, Das, Sudarshan, and Anderson, 1971; Menaker and Navia, 1973). However, whereas maternal body-weight begins to plateau around the 12th day, maternal food intake continues to increase. Ota and Yokoyama (1967a) reported a steady increase in maternal food-intake in the first two postpartum weeks, followed by an abrupt increase in the third week up to weaning at 21 days, after which food-intake dropped. Cotes and Cross (1954) found that ligation of the galactophores did not affect this pattern of food-intake or maternal body-weight gain in mothers who were suckled to foster litters. They concluded that suckling stimuli were responsible for stimulating food-intake in excess of that required to support maternal metabolism. The increase in food-intake was not,

therefore, related to increased metabolic demands brought about by satisfying the food requirements of the growing litter as the ligation procedure results in involution of the mammary glands although the litter may still suckle.

It appears that increases in the circulating level of prolactin in the postpartum period (Amenori, Chen, and Meites, 1970) consequential to the suckling stimulus may mediate the increase in food-intake over and above that required to support the normal metabolism of the mother. Leon (1974) has ascribed a unique significance to this enigmatic increase in maternal food-intake. He suggests that the extra food material is used to manufacture an increased amount of fecal material (caecotrophe) at a critical stage in pup development. The caecotrophe may serve the following functions: (i) as an olfactory cue (pheromone) which ensures continuing contact between the mother and her pups in the transition stage from the time the pups are completely dependent upon the mother (first two weeks) until the time they assume autonomy (end of third week) (Leon and Moltz, 1971; Leon and Moltz, 1972); (ii) as a transitional "baby-food" during this same period, and perhaps involved in subsequent diet selection by the pups (Leon, 1974).

Although Ota and Yokoyama (1967a) found that after weaning maternal body-weight returned to a level which would be expected had the rats not undergone pregnancy, their suggestion that the body-weight changes of pregnancy and lactation may be superimposed upon normal growth is

open to question. In any event, the 10 gm. increase they observed in maternal body-weight at weaning over body-weight at the start of pregnancy, is less than the weight increase that would be expected in a virgin rat over a comparable six-week period (see Freudenberger, 1932).

The increase in body-weight of the pregnant rat in the first two weeks of gestation is probably due to a genuine growth response to the rising levels of progesterone that have been reported (Hashimoto, Henricks, Anderson, and Melampy, 1968) and similar to that of non-pregnant females receiving exogenous progesterone (Hervey and Hervey, 1967). Over the last week of pregnancy, the increase in maternal body-weight can be almost completely accounted for by the increase in weight of the products of conception.

That the absolute gain in weight in the heavy and light groups during pregnancy was identical and litter size comparable in both groups, suggests that maternal absolute weight gain was determined by litter-size or, more specifically, perhaps by increases in endogenous progesterone secretion in the first half of pregnancy. In the postpartum period, however, the light mothers retained more weight than the heavy mothers and by the postweaning plateau the original difference of 33 gm. at the start of pregnancy was reduced to 20 gm. A possible explanation for this may lie in the fact that in the postpartum period both groups of mothers received an equivalent suckling stimulus from eight pups, and the corresponding increase in postpartum progesterone (Grota and

Eik-Nes, 1967; Tomogane, Ota, and Yokoyama, 1969) and prolactin (Everett, 1966) would presumably therefore be comparable in both groups. Contingent relationships have been described between the number of suckling pups and progesterone secretion (Eto, Masuda, Suzuki, and Hosi, 1962) and prolactin secretion (Grosvenor, Mena, and Schaefgen, 1967). In mothers of lower body-weight the amount of these hormones per unit body-weight would be greater than in mothers of higher body-weight, inducing by the postweaning stage a higher overall weight gain in the light group compared with the heavy group.

An alternative possibility is that, in the light group, there exists a higher potential for weight-gain as these animals are further from their asymptotic body-weight than those of the heavy group. These two possibilities could be investigated by using mothers of comparable body-weight at the start of pregnancy who give birth to litters of comparable size. Following parturition, one group could be given a large number of pups and the other group a small number. If the first hypothesis above were correct, the mothers receiving a greater suckling stimulus from the larger litter would be expected to incur a higher overall gain in weight by the postweaning stage than the mothers with small litters. The second possibility would be excluded as both groups of mothers are at the same stage in their normal growth curve.

A study by Ota and Yokoyama (1967b) has a direct bearing on the suggestion above, since body-weight increases in mothers rearing

different litter-sizes were investigated; it does not, however, resolve which of the two possibilities above is correct. From their data, a mean difference of 20 gm. in maternal body-weight is apparent between the group rearing litters of 12 and that rearing a litter of 2 on Day 1 of lactation. It is unlikely that similar differences existed at the start of pregnancy. No data were provided concerning the number of offspring born to the various mothers prior to the postpartum manipulation of litter-size, nor with regard to the postweaning residual weight gains in the groups as maternal body-weight was studied only as far as Day 15 of lactation.

Maternal Behaviour

Before embarking on a discussion of the present findings on maternal behaviour, some comment is in order concerning the study of maternal behaviour and its three basic aspects: (i) initiation, (ii) maintenance, and (iii) modulation.[1] Since the early pioneer work on the subject (Kinder, 1927; Wiesner and Sheard, 1933) the bulk of experimental work with the rat has been directed towards the first two, the systematic analysis of factors underlying the initiation of maternal behaviour following parturition and its subsequent maintenance in the postpartum

[1] The third aspect has previously been described as "decline" (see Rosenblatt, 1969); however, as the findings from the present study and others (Grota and Ader, 1969; Ader and Grota, 1970) show that decline (measured as nest-time) occurs through the first two weeks, the term "modulation" may be more appropriate and will be used here.

period. A large number of studies on these two aspects of maternal behaviour have appeared in the literature over the last fifteen years and a wealth of information has been forthcoming (for reviews see Rosenblatt, 1969; Moltz, 1971). The general question posed in these studies is: Is the mother being maternal or not? The answers have usually been provided by qualitative observations on retrieving, nest-building, nursing, etc. Only infrequently were efforts made to quantify maternal behaviour such that individual differences might be assessed (e.g., Seitz, 1958). Indeed, as Deitchman (1970) notes, this approach appears to have resulted in individual variability being overlooked rather than it being the subject of investigation.

In the experimental analysis of the effects of early experience, however, it is becoming clear that more attention should be directed towards maternal involvement, and the possibility recognised that changes in the interaction between the mother and her litter may play a critical mediating role in the elaboration of many of the phenomena that have been described (see Plaut, 1970; Russell, 1971). For example, in a recent study of undernutrition in the rat, in which maternal and litter effects were controlled, there was little evidence of the behavioural deficits in adulthood that have frequently been reported where such procedures have not been followed (Slob, Snow, de Natris-Mathot, 1973).

Clearly, there is a need for a more detailed knowledge of maternal behaviour; besides establishing whether or not a mother is maternal

a quantitative assessment of maternal behaviour is also required. In addition there is also a need for information concerning those factors which modulate maternal behaviour, e.g., what initiates a nursing period and what brings it to a close? How do these control mechanisms change through the postpartum period? In the discussion which follows an attempt will be made to integrate some of the more recent findings on maternal and neonatal behaviour from these two standpoints of quantification and modulation.

Nest-time:--On the basis of representative sampling techniques it has generally been concluded that the amount of time a mother rat spends on the nest is relatively constant in the first two postpartum weeks (Rosenblatt and Lehrman, 1963; Moltz and Robbins, 1965; Rosenblatt, 1969, Moltz, 1971). The results of Experiment Va (based on a three-hour daily observation period) are consistent with this view. The data from the present study, together with those from others in which a continuous recording system has been employed (Grota and Ader, 1969; Ader and Grota, 1970), however unequivocally demonstrate that nest-time undergoes a progressive and orderly decline through this period. An explanation for the absolute differences observed between the results obtained with the present system and those obtained by Grota and Ader (1969) was provided in an earlier discussion (Experiment VI). In a study where the sampling period was extended to six hours (Experiment Vb), and where sampling was conducted in both phases of

the light-dark cycle (Deitchman, 1970), a decline in nest-time was also observed in the first two postpartum weeks. Before the differences between heavy and light groups in the present study are discussed, the possible significance of this decline in nest-time will be considered.

The first question is: Why, when the mother is the sole source of nutrition for her litter, does her nest-time decline at all? It might be expected, for example, that if the main purpose of the mother being on the nest is to provide nourishment for the pups, then the amount of time she spends there should remain at a fairly high and constant level during these two weeks. The absolute gain in litter-weight rises steadily in the first ten days and is relatively constant for the next five (Ota and Yokoyama, 1967a), thus, the litter is at least maintaining its growth rate and presumably its nutritional demands despite the decline in nest-time. Several possibilities for the decline in nest-time are suggested: (i) as the pups grow older and stronger, they become more efficient at removing milk from the mother; (ii) as well as the suckling stimulus, visual, acoustic, and tactile stimuli from the pups progressively recruit a greater galactopoietic[1] response from the mother, and perhaps the latency to first milk-ejection and the inter-milk-ejection interval both shorten over time; (iii) changes in the hormonal state of the

[1]The term "galactopoiesis" is used here to mean the enhancement of an already established milk secretion, and not, as it is sometimes used, to indicate the maintenance of lactation (see Cowie and Tindall, 1971).

mother during this time produce a progressive inhibition of nursing behaviour; (iv) the purpose of the mother being on the nest has important connotations other than that of providing nourishment for the pups. These possibilities will now be discussed in turn.

(i) Several studies provide evidence which bears indirectly on the first of these possibilities, that the vigour of suckling increases with advancing age of the pups so that milk is removed more rapidly from the mother. Bateman (1957) ascribed the more rapid growth seen in the larger individuals of a litter to "passive competition" i. e., that differential growth rates do not occur simply because smaller individuals are actively ousted from a nipple position by larger individuals, but because the bigger individuals are able to feed faster during a nursing period. If this is the case, then, as all individuals get bigger they will feed faster as lactation progresses and this may result in the decline in nest-time. Grota (1973), using the dual-chamber continuous recording system, found that age of pups at fostering was a significant determinant of the amount of time the mother spent with the litter. Kumaresan, Anderson, and Turner (1967) studied the weight gain of litters in a 30 min. nursing period following a 10 hr. separation from the mother on Days 14, 16, 18 of lactation. Their results generally show that milk yield was greater as lactation advanced and thus as the pups grew bigger. This study suggests that with advancing age pups become more efficient at removing milk from the mother, although galactopoietic changes in

the mother during this time, and the other possibilities of (ii) (see below) may not be excluded. It may be possible to answer the question by separating a group of mothers all nursing litters of equal size at a given lactational age, anaesthetising them, and administering oxytocin. Litters of equal size, but of differing age, could then be put to the mothers and the respective weight-gains of the litters recorded after a given time-interval had elapsed following attachment of the pups to the nipples. The use of anaesthesia would eliminate the possibility that mothers would respond differentially to litters of differing age (Rosenblatt, 1965; Grosvenor, Maiweg, and Mena, 1970; Grota, 1973) and the administration of oxytocin would effect more complete milk withdrawal and therefore greater litter weight-gain (Kumaresan and Turner, 1966) which would optimise the likelihood of observing differences between the litters.

(ii) The second possibility, that there are galactopoietic changes, and changes in the temporal characteristics of milk-ejection, during the course of lactation, does not seem to have been investigated directly although, again, there are several studies which offer some indirect evidence. For example, an increase in mammogenesis (mammary gland development) in the course of lactation, is suggested by increases in mammary DNA content which are taken to reflect cellular increases in the mammary gland (see Schmidt, 1971), and increases in the amount of ethanol extracted mammary tissue (Tucker and Reece, 1963).

Functional increases in the mammary gland would be expected to parallel these structural increases, and hence it may be reasonably assumed that corresponding galactopoietic increases also occur. Several studies have reported a progressive increase in milk-yield through lactation (Brody and Nisbet, 1938; Grosvenor and Turner, 1958; Fuchs, 1969; see also Walsh and Tucker, 1972) whereas others have reported increases up to about Day 10 (Park and Nowosielski-Slepowron, 1972) or Days 11-13 (Gustaffson, 1948) and a decline thereafter. That the gonadal steroids, progresterone and estrogen, in synergism with prolactin and growth hormone, may be responsible for these increments in structure (Meites, 1966) accords well with the findings of increases in the circulating levels of progesterone (Grota and Eik-Nes, 1967; Tomogane, Ota, and Yokoyama, 1969) and prolactin (Amenomori, Chen, and Meites, 1970; Simpson, Simpson, Sinha and Schmidt, 1973) in early lactation. Other studies on the stimulus characteristics of prolactin release during the course of lactation are of particular interest.

Grosvenor (1965) found that if mothers were separated from their litters for 10 hr. on Day 14 of lactation, and then replaced with their litters for 30 min. but prevented from nursing, a reduction in prolactin concentration of the maternal pituitary occurred comparable to that which occurs following nursing. It was concluded that exteroceptive stimuli from the litter were responsible for the effect and that the suckling stimulus was not needed at this stage of lactation for the release

of prolactin. Subsequently, it was suggested that olfactory stimuli from the litter were primarily responsible for prolactin release, although in mothers chronically deprived of their sense of olfaction, visual stimuli could take over (Mena and Grosvenor, 1971). In a study in which mothers were thelectomised (surgical removal of nipples) the output of prolactin was sustained through lactation, as indicated by the deciduoma response and the suspension of ovarian cyclicity (Moltz, Levin, and Leon, 1969b). It was concluded that in the absence of a suckling stimulus, visual, acoustic, olfactory, and perhaps tactile stimuli from the pups were responsible for the sustained release of prolactin. However, following observations that a 30 min. exposure to pups without suckling did not result in a fall in maternal pituitary prolactin on Day 7 of lactation, Grosvenor, Malweg, and Mena, (1970a) proposed that the effects observed by Moltz, Levin, and Leon (1969b) were due to a more specific tactile (para-suckling) stimulation than had been proposed. It was argued that receptors both in and around the mammary glands could have been stimulated by "nuzzling" of the pups and that this mediated the continuing prolactin release. Findlay's work (1966) on the rabbit mammary gland, in which discharges in mammary afferents were recorded in response to a variety of mechanical, thermal, and chemical stimuli, was cited in support of this view. It was concluded that suckling was the primary stimulus for prolactin release in the early stages of lactation, and that in the second week generalisation occurred so that

other exteroceptive stimuli could come to elicit the response. Further evidence of such associative learning was provided by the observation that whereas primiparous mothers did not show a significant depletion of pituitary prolactin on non-nursing exposure to pups on Day 7 of lactation, mothers undergoing a second lactation did at this time. It was later demonstrated that on Day 21 of lactation the prolactin response had generalised to the point at which the presence of other lactating mothers and their litters, housed in the same room, was capable of eliciting it (Mena and Grosvenor, 1972). Interestingly, it was observed in a recent study (Grosvenor and Mena, 1973) that this generalised response did not result in a corresponding stimulation of milk secretion if the mother was exposed to her own pups 3-4 min. before exposure to the other mothers and their litters. Milk secretion was similarly inhibited by the presence of the mother's own pups underneath her even though there was a significant release of prolactin in response to the exteroceptive stimuli from these pups. The significance of this inhibitory mechanism will be discussed later under possibility (iii).

The results of these studies indicate that in the first week of lactation, suckling, or even "para-suckling," is the primary stimulus for the release of prolactin. Between the end of the first week and the beginning of the third, there is a generalisation of the suckling stimulus to other pup-related exteroceptive stimuli, and by the end of the third week, a further generalisation has occurred such that the presence of

other lactating mothers and their litters may elicit the response. To summarise, structural and presumably functional increases in the mammary glands in the course of lactation develop initially as a result of rising titres of progesterone and prolactin. The frequent suckling stimulus in the early stages of lactation, increases in its strength (see (i) above), and the later generalisation to exteroceptive stimuli, are responsible for a sustained release of prolactin, which, through its galactopoietic effect, may give rise to a condition of increasing lactatory preparedness and potential such that milk is produced more readily in a given time.

The discharge of milk involves the other possibilities mentioned in (ii), that the latency to milk-ejection (ME) and the interval between MEs may be shortened through lactation, although little work has been done on this important aspect of nursing. The release of milk from the mammary gland is either passive (removal) or active (ejection). In milk removal, there is a passive withdrawal of only a small proportion of milk from the large sisterns and sinuses, whereas milk-ejection, resulting from the action of oxytocin on the contractile myoepithelial cells surrounding the alveoli and small ducts, achieves the active expulsion of a greater proportion of the milk.

Wakerley and Lincoln (1971) demonstrated that the pattern of ME in the rat was intermittent and associated with the pulsatile release of oxytocin. Increases in the firing rate of paraventricular neurons were

observed prior to oxytocin release (Lincoln and Wakerley, 1972); the interval between this increase in the electrical activity of the neurosecretory cells and the release of oxytocin at ME was found to be in the order of 15-20 sec. and although not associated directly with the suckling stimulus, did not occur in its absence (Wakerly and Lincoln, 1973).

In a detailed study of intramammary pressure, temporal characteristics of ME, milk yield, and pup behaviour during nursing on the 10th day of lactation (Lincoln, Hill, and Wakerley, 1973), the latency to first ME following the onset of suckling was found to be about 12 min. and subsequent ME intervals were in the order of 10 min. Observations of the pups revealed a vigourous suckling on being first put to the nipple followed by a subsidence in activity. During this period (before the first ME) some milk was removed. The occurrence of the first ME was not associated with any prior overt change in intramammary pressure nor in pup behaviour. The commencement of ME resulted in a characteristic "stretch" response simultaneously in all pups, during which there was strong vigourous pulling on the nipples, and which resulted in a appreciable amount of milk being obtained. After about 8-12 sec. many pups detached themselves from the nipples, sought other nipple locations, and within 20-30 sec. had re-attached themselves to nipples. The amount of milk obtained in subsequent MEs was progressively reduced. The afferent part of the ME reflex was shown to be related to two

factors: (i) as the degree of mammary engorgement increased, the latency to first ME decreased and the frequency of subsequent MEs increased, and (ii) when the number of suckling pups was reduced by half, an increase in the latency to first ME was observed and the interval between MEs showed some increase.

These new observations raise several important points in the light of the present discussion. Firstly, if as was suggested above, there are galactopoietic changes through lactation and more milk is available at later stages, and as the frequency of nursing drops slightly through lactation, then a relative increase in mammary engorgement should occur between nursing periods as lactation proceeds. This may result in a systematic change in the temporal characteristics of ME. In the later stages of lactation, however, a positive feedback mechanism may operate to further reduce nursing time. Reddy, Donker, and Linnerud (1964) have demonstrated that milk secretion rate falls as the interval between nursing periods increases. Lengthening the interval between sucklings also results in a reduction in the milk secretory response to prolactin (Grosvenor, Malweg and Mena, 1970b) and in a reduced release and reaccumulation of prolactin (Grosvenor and Mena, 1971). Secondly, that the latency and interval characteristics of ME are related to the number of pups suggests that other contingencies between these parameters and pup-related stimuli exist through the course of lactation (cf. Dels, 1968), perhaps interrelated with the characteristics of prolactin release.

If either or both of these possibilities are determinants of systematic changes in the temporal and frequency properties of ME, they may also determine the decline in nest-time. As the occurrence of individual MEs is readily apparent from the overt behaviour of the pups, it would be a relatively straightforward task to examine the possibility of such changes during the course of lactation.

(iii) The third possibility, that hormonal changes in the mother are involved in the modulation of maternal behaviour, has been investigated in several studies. On the basis of the observation that maternal behaviour appears immediately following parturition, and that as parturition approaches there is a substantial decrease in levels of circulating progesterone (Grota and Eik-Nes, 1967; Kuhn, 1969) it was suspected that progesterone might play an inhibitory role in the regulation of maternal behaviour. Moltz and Wiener (1966) ovariectomised primiparous and multiparous rats in late gestation and delivered litters by caesarean section. In the immediate postpartum period all of the multiparous mothers showed normal maternal behaviour towards their pups whereas this behaviour was delayed until after postpartum Day 3 in 50% of the primiparous mothers. Similar results were obtained in a later study in which progesterone was administered over the last four days of gestation (Moltz, Levin, and Leon, 1969a). It was suggested that progesterone exerted an inhibitory influence on maternal behaviour by preventing

the uptake of prolactin in the brain; as the progesterone titer fell in late gestation, prolactin was dis-inhibited and maternal behaviour emerged under the coactive influence of prolactin and estrogen. The failure of mothers to manifest maternal behaviour was attributed to the disruptive effects of progesterone on the coactive influence of progesterone and estrogen. The finding in both studies that some of the primiparous, and all of the multiparous mothers, behaved maternally was explained in terms of a lowered threshold to hormonal stimulation. In the case of primiparous mothers, it was attributed to individual variability, and, for the multiparous mothers, to their previous breeding history. In support of these conclusions it has recently been demonstrated that progesterone may inhibit the synthesis of prolactin (Chen and Meites, 1970; Simpson, Simpson, and Kulkarni, 1973), and that previous breeding experience lowers the threshold for prolactin release to exteroceptive stimuli in early lactation (Grosvenor, Malweg, and Mena, 1970a).

The influence of exogenous progesterone on maternal behaviour in the early postpartum period was investigated by administering progesterone twice daily over four days commencing two days postpartum (Moltz, Levin, and Leon, 1969a). It was reported that mothers so treated nursed, maintained nests, and retreived their young, and the conclusion was reached that progesterone was not involved in the modulation of already-established maternal behaviour. The results of this study were later discussed by Moltz (1971) who suggested that the failure

of progesterone to influence maternal behaviour was not surprising in view of the report that endogenous titers of progesterone normally increase in early lactation (Grota and Eik-Nes, 1967). Thus, the exogenous progesterone was simply adding to the levels of the increasing endogenous progesterone, and, as it was believed that nursing did not decline in the first three postpartum weeks (Moltz and Robbins, 1965), there was little reason to believe that progesterone did have a modulatory influence on maternal behaviour during this time.

However, the finding that nursing behaviour does show a substantial decline in early lactation (Grota and Ader, 1969; Ader and Grota, 1970; Deitchman, 1970; Experiment Vb of this thesis; present study) coupled with the increasing levels of progesterone in early lactation (Grota and Eik-Nes, 1967; Tomogane, Ota, and Yokoyama, 1969) and the suggested inhibitory influence of progesterone discussed above, indicate rather that progesterone may exert a progressive inhibition on maternal behaviour through the first half of lactation. The proposed inhibition of the onset of lactation during pregnancy by high levels of progesterone (Meites and Turner, 1942; Cowie and Folley, 1961) raises the possibility that increasing titers of progesterone in early lactation may induce a second phase of inhibition, although other reports (Reece and Bivins, 1942; Lyons, Li, and Johnson, 1958) have suggested that progesterone actually enhances lactogenesis. A resolution of this conflict has been proposed by Herrenkohl and Lisk (1973) who suggest

that progesterone inhibits the release rather than the secretion of milk. They observed that progesterone treatment of rats in late gestation, or in late gestation and in the early stages of lactation, did not significantly reduce milk secretion but did result in a significant reduction in milk-ejection in both groups (the mother was scored positive if the pups showed stomach distension with milk at the end of a 15 min. test nursing period, milk-ejection proper was not therefore measured and the result could have been due to milk removal). Interestingly, the incidence of maternal crouching over the young was significantly reduced only in the group in which progesterone was given postpartum. This confirmed an earlier finding that postpartum injections of progesterone suppressed nursing behaviour (Herrenkohl, 1972). In a more recent study, progesterone administration in late gestation was found to increase the duration of subsequent nursing (Herrenkohl, 1974).

To summarise these studies, the balance of evidence seems to weigh in favour of progesterone involvement in the modulation of maternal behaviour. The initiation of maternal behaviour occurs at a time when progesterone levels are low, and its onset can be delayed by the administration of progesterone to primiparae. Nursing time shows a progressive decline as the levels of progesteronr rise, and two studies have demonstrated a suppression of maternal behaviour when progesterone is administered in the postpartum period. There remains the problem that maternal behaviour has been assessed on the basis of relatively

short observation periods which may not be sufficiently representative to provide information on the quantitative changes in maternal behaviour that may result from progesterone treatment. Work in progress (Brown, Leon, and Croskerry) is examining the effects of gonadal and adrenal steroid depletion on maternal behaviour, using the continuous recording system described (Experiment VI).

Several studies have investigated the possibility that prolactin, which is essential for lactation, is involved in the regulation of maternal behaviour. On the basis of the observation that a significantly higher proportion of mothers, deprived of their litters on the first postpartum day and treated with oxytocin and prolactin for 10 days, showed maternal behaviour compared with mothers similarly deprived of their litters but without hormone treatment, Rosenblatt (1965) suggested that prolactin and oxytocin were involved in the maintenance of the maternal condition. Although a galactopoietic role has been ascribed to oxytocin (review in Cowie and Tindall, 1971), the pulsatile suckling-dependent nature of its release (Wakerly and Lincoln, 1971; Lincoln and Wakerly, 1972; Wakerley and Lincoln, 1973) presents technical difficulties in the estimation of representative titres through lactation. Less variability presumably occurs in the release of prolactin, and two studies have measured prolactin levels during lactation. Amenomori, Chen, and Meites (1970) reported high levels of serum prolactin in the first week of lactation with a peak value on Day 6, followed by lower values on

Days 15 and 23. In a study of plasma levels, essentially the same pattern was reported with a peak on Day 5 and progressivly lower levels on Days 10, 15, and 20 (Simpson, Simpson, Sinha, and Schmidt, 1973). Numan, Leon, and Moltz (1972) investigated the suggested involvement of prolactin in maternal behaviour (Rosenblatt, 1965) by giving daily postpartum injections of ergocornine, a drug previously shown to suppress the release of prolactin. Effective suppression of prolactin was confirmed by an almost non-existent deciduoma response at 10 days following uterine trauma at Day 5, and by a suppression of milk production as measured by litter weight change. The inhibitory effects of ergocornine on luteal activity were alleviated by twice daily doses of prolactin; the deciduoma response in the ergocornine + prolactin group was not significantly different from that of the group receiving the vehicle alone. An amelioration of the effects of ergocornine on litter-weight was also achieved through prolactin replacement. Despite the clear effects of ergocornine on prolactin release, no significant effects on maternal behaviour were observed in the tests used. Standard tests of retreiving and nest-building were administered, together with observations of total time spent nursing in either 10 or 30 min. observation periods. The authors' conclusion that prolactin was not essential in maintaining already-established maternal behaviour receives some support from other studies. For example, from the relationship between the suckling stimulus and prolactin release, it would be expected that the quantity of prolactin

released would be less in a litter of few pups compared with a litter of many, and this appears to be the case. Amenomori, Chen, and Meites (1970) reported a significant reduction in serum prolactin following reduction of litter number. However, several studies have described an inverse relationship between the amount of time a mother spends with her litter and the number in the litter (Seitz, 1954; Grota and Ader, 1969; Hofer and Wiener, 1971; Grota, 1973). If endogenous prolactin levels in a mother nursing a small litter are consistently lower than those of a mother nursing a large litter, then the relationship between prolactin levels and maternal behaviour is opposite to that predicted by Rosenblatt's hypothesis (1965).

A difficulty with the Numan, Leon, and Moltz (1972) study, as with the studies of progesterone, and with others that have suggested a non-essential role for estrogen (Rosenblatt, 1969) and the adrenal steroids (Thoman and Levine, 1970), is that conclusions based on short observation periods are limited. In broad terms, it can certainly be stated that such hormonal manipulations do not grossly affect maternal behaviour and therefore these hormones can be considered "non-essential;" indeed, they may well prove to exert little influence on maternal behaviour. However, without the use of more extensive observations on the daily, and circadian pattern of maternal behaviour through lactation, no definitive statement can be made with regard to its modulation by progressive shifts in the hormonal state of the mother.

A specific example of an hormonal inhibitory mechanism for nursing was suggested in the study of Grosvenor and Mena (1973) mentioned earlier. It was demonstrated that although there was a widespread generalisation of the prolactin response to exteroceptive stimuli in late lactation, milk secretion could be blocked by exposure of the mother to her own pups. The administration of an adrenergic blocking agent, phentolamine, was subsequently found to dis-inhibit the effects of exposure to the litter by allowing a normal secretion of milk in response to the prolactin released at exposure. It was proposed that the emission of olfactory and perhaps auditory stimuli from the pups induced catecholamine release in the mother which achieved peripheral inhibition of prolactin-induced milk secretion, and it was suggested that this mechanism may act to reduce milk secretion in late lactation and thereby facilitate the weaning process. Although this mechanism (perhaps in conjunction with the cessation of maternal pheromone release - see earlier discussion) may act to reduce contact and dependency of the young on the mother around the end of the third week of lactation, it is unlikely that it is involved in the earlier decline of nursing as there was no evidence of such peripheral inhibition of milk secretion at Day 14 of lactation.

In conclusion of this discussion of the possible hormonal control of maternal behaviour it should be pointed out that, in addition to its effects on prolactin release, the suckling stimulus may also result

in the release of GH, TSH, ACTH, and MSH (reviewed by Nicoll, 1971; see also Cowie and Tindall, 1971) and the possibility remains that changes in the quantitative properties of the suckling stimulus through lactation, with perhaps the development of associative learning responses to exteroceptive stimuli similar to those which have been described for prolactin release, produce changes in the secretory pattern of these hormones during lactation which may result in a modulation of maternal behaviour.

(iv) The fourth possibility will now be considered, that maternal presence has a functional significance additional to that of nursing. It has been established that nursing is not the singular purpose of the mother being in the nest; in addition to providing nourishment for her pups, the mother also grooms them, facilitates the development of elimination reflexes, and provides protection from predators (Wiesner and Sheard, 1933; Rosenblatt and Lehrman, 1963; Rosenblatt, 1965). In addition to this general caretaking role, the mother also serves the other important function of maintaining the temperature of the pups within the necessary limits for survival.

The ability to regulate body-temperature at birth depends upon the proportion of body-fat (Thomas, 1911; cited in Blaxter, 1961). Compared with other newborn mammals, the rat has a relatively low proportion of body-fat amounting to a little over 1% (Widdowson, 1950). The subsequent deposition of fat insulates against heat-loss, and this together with the

development of the epidermis, fur-covering, and vasomotor mechanisms, permits homoiothermia to be achieved. Although it is frequently stated that the rat is poikilothermic at birth, several studies have demonstrated an active metabolic response to a change in ambient temperature (Gelineo and Gelineo, 1951; Taylor, 1960; Poczopko, 1961); the response is very weak at birth, improves following suckling, and progresses rapidly from 6 hr. onwards (Taylor, 1960). Poczopko (1961) observed that the body-temperature (almost identical to skin-temperature) of a 1-day old pup followed a rise or fall in ambient temperature and, after equilibrium was established at any temperature in the range 20° - 30° C., pup temperature was always above ambient. Prior to 4 days of age, temperature changes in the range 25° - 35° C did not produce any visible changes in the constriction or dilation of blood vessels in the skin; vasomotor mechanisms for the conservation and dissipation of heat subsequently developed between 4-20 days. In the basis of these observations, it was suggested that the neonatal pup be classified as "heterothermic" in that its body-temperature at normal activity was greater than ambient but lower than that of the adult. The term "ecto-thermic" (see Young, 1962) is also appropriate in that the pup depends largely upon an external source to maintain body-temperature within reasonable limits.

Homoiothermia develops gradually and there is general agreement that it is attained sometime in the third week (Hahn, Krecek, and Kreckova,

1956; Adolf, 1957; Taylor, 1960; Poczopko, 1961; Okon, 1971; Noirot, 1972). The data of Baccino (1935) show that albino rats select a temperature of about 30° C on Day 2 and subsequently select lower temperatures. Gustaffson (1948) placed pups on a thermogradient (20° - 45° C) to determine temperature preference; at ages 0, 5, 11, and 17 days the values were 37°, 35°, 33°, and 24° C respectively. On the basis of observations on oxygen consumption at various temperatures, Taylor (1960) concluded that there was a shift in neutral zone (temperature range in which there is no change in oxygen consumption rate) with age. In the first six days of life it was estimated to be in the range 33°-38° C, and was slightly lower with a wider range of variability at three weeks. This is contrasted with Poczopko's (1961) "quasi-normal" development of pup temperature, which appears to have been based upon the body-temperature attained after 40 min. at a room temperature of 26° C. These were 29°, 30°, 31°, 33°, and 35° C at ages 1, 4, 8, 12, and 16 days respectively. While Taylor's measure serves to indicate the temperature range, at a particular age, in which the pup is probably best-suited in terms of metabolic homeostasis, that of Poczopko (see also Okon, 1971) shows the development of the pup's ability to conserve heat against a standard gradient, and hence reflects the development of homoiothermia.

Various estimates of the nest-temperature, with the mother present, have been made. According to Gelineo and Gelineo (1951) and Allin

and Banks (1971) it is about 35° C, whereas Moltz (1971) states that the mother must maintain the nest at approximately 100° F (38° C). Taylor (1960) estimated nest-temperature at 32° C but did not indicate whether the mother was present or not. If the estimate of 35° C is accepted, which lies about mid-way in the range of neutral zone, and room temperature is taken to be about 22° - 24° C, there will be a gradient of approximately 12° C when the mother is absent from the nest.

Gelineo and Gelineo (1952) reported that the core temperature of a 2-day old rat fell 11° C over 30 min. of maternal absence, and the data of Hutchings (1963) shows that the rectal temperature of 1-2 day old pups, placed on wood shavings at 23° C, dropped from 35° C to 27° C in 20 min. These estimates appear to be a little higher than would be expected on the basis of extrapolation from Poczopko's (1961) data. The different values obtained are probably due to the contact medium on which the pup is placed as Hutchings (1963) observed that at 23° C pup temperature dropped from 35° C to 30° C in 7 min. on wood shavings but took only 3 min. to drop by the same amount in a metal can at 23° C. At 12 days of age, when heat conservation mechanisms are fairly well developed, significant increases in pup temperature occurred with nursing, and in other litters exposed to prolonged maternal absenteeism body-temperature fell in several cases to less than 33° C and as low as 31° C (Hofer and Grabie, 1971). Others have reported a decline in pup temperature associated with maternal absence (McIver, Jeffrey, Stevenson, and

Nielson, 1963).

Although these studies clearly demonstrate the thermoregulatory role of the mother, the data tend to exaggerate the temperature loss that pups will suffer under normal conditions. As Small (1899) observed in his classic observations on development of the rat, when the mother is away from the nest the pups demonstrated their imperative need for warmth by huddling together in a heap. Hutchings (1968) points out that the intact litter may be considered as a biological aggregate which effectively reduces the ratio of surface area to body-volume and therefore minimises heat loss during maternal absence from the nest. The amount of heat loss that will be incurred at a given age will depend not only on the duration of the mother's absence but also on the characteristics of the nest and the number of pups in the litter. When the litter is small, the effective reduction of surface area to body-volume is less than for a large litter and heat conservation is therefore less efficient. It is worthy of note that females rearing small litters maintain more complete covering of the young with nest material than do females rearing large litters (Draper, 1968; see also Barnett and Mount, 1967). Thus, although the litter will suffer some loss of heat when the mother is away from the nest, it would not be as great as that occurring under conditions of individual isolation. Other observations also involve the mother in a thermoregulatory role: several reports have demonstrated that the mother strives to regulate the temperature of the nest by selecting

a suitable thermal location (Kinder, 1927; Sturman-Hulbe and Stone, 1929; Gelineo and Gelineo, 1951); the quality of the nest is determined by ambient temperature (Kinder, 1927; Gelineo and Gelineo, 1951); and surgical interference with maternal temperature regulation (see Rosenblatt and Lehrman, 1963) changes nest-building behaviour.

It may be asked what the consequences are for the pups of these periodic losses of temperature associated with maternal absenteeism. Schaeffer (1968) and Hutchings (1968) have suggested that even small excursions in the body-temperature of the pup may critically affect the many metabolic and enzymatic processes that are temperature-dependent, and therefore may exert a profound influence on development. Gelineo and Gelineo (1951) demonstrated that the development of homoiothermia is determined by ambient temperature during the first two weeks of postnatal development. The suggestion by Schaeffer (1963) and others that temperature changes in early life may mediate the effects of early handling will be discussed in more detail later.

It appears from the studies reviewed this far that the presence of the mother in the nest serves a critical function in addition to that of providing nourishment. That the decline in nest-time appears to reciprocate the development of thermal regulation by the litter suggests that temperature regulation may be directly involved in the modulation of maternal behaviour. However, the effective operation of such a system would require some mechanism by which the mother is made

aware of, and responsive to, the temperature state of her litter. Two such mechanisms will now be discussed, one coming from the pups and the other from the mother, which together may be involved in the acute and overall regulation of nursing time.

It has recently been demonstrated, that under conditions of thermal and tactile stress, neonatal pups emit ultrasonic calls (review by Noirot, 1972) which are capable of eliciting maternal searching and retrieval behaviour (Sewell, 1970; Allin and Banks, 1972). Generally, the functional value of this communication system has been interpreted in terms of its survival value for the individual pup who becomes isolated from the nest, and the design of experiments has been directed accordingly. Typically, a pup is taken from the nest, placed in isolation at a particular temperature under specified conditions, and the parameters of acoustic emissions measured. This procedure, although sometimes confounding thermal, tactile, and perhaps other stimuli (see Noirot, 1972) that induce calling, does reasonably examine the isolation condition, and there appears to be little doubt that the system of communication between pup and mother serves the important function of protecting the pup from the deleterious consequences of prolonged isolation.

Little emphasis has been placed upon the ability of the pups to modulate maternal behaviour under normal conditions, i.e., by calling the mother to the intact nest to alleviate hunger or thermal distress although this function may have been implicitly assumed in many

of the studies that have been concerned with the phenomenon. Indeed, it seems unlikely that such a mechanism should have evolved solely for the purpose of minimising the isolation of individuals, especially as in the natural habitat the nest is of a more compact and protective design (see Calhoun, 1962) in which the frequency of occurrence of isolation would probably be minimal. Logically, it would appear that such a system might play a more fundamental and ongoing role than has hitherto been suggested. Allin and Banks (1971) suggested that the return of the mother to the nest might ameliorate pup distress but no explicit formulation of the conditions under which this might occur was made, and again the impression is conveyed that distress calls only modulate maternal behaviour under conditions of extremis. Thus, it is not presently known if, after some threshold of temperature loss has been exceeded following maternal absenteeism, the pups might emit distress calls in unison, which would be of sufficient intensity to bring the mother to the nest. Changes in the intensity and rate of calling occur with advancing age and therefore with the development of homoiothermia (Okon, 1971) which indicates that, as homoiothermia develops, the time taken to reach this critical threshold for emission would increase, and the interval between successive nursing periods should therefore decrease. The data from the present study show an increase in the relative frequency of longer between-nursing intervals as lactation proceeds.

This hypothesis could be investigated by determining the characteristics

of sound emissions from the intact litter, at the same time monitoring litter temperature and maternal behaviour. It may be that such acoustic signals from the litter do not always induce the return of the mother to the nest, learned components of the mother's behaviour may come to play an increasing role through the course of lactation. The mechanism may only operate at earlier stages, and perhaps only when the duration of maternal absence exceeds some limit. Further, the type of arousing stimulus (thermal, tactile, hunger, humidity) that causes the pup to emit sounds may assume differential importance at different stages of development (see Bell, 1974). Also, the possibility should not be excluded that, in the early stages of lactation when the pups emit calls reflexively in response to physiological distress, they come to recognise the temporal contiguity between this behaviour and the mother's attendance. In the later stages of development, when the pups become less vulnerable to the vicissitudes of the environment, the sounds may be emitted in an operant fashion to obtain maternal reinforcement. Some evidence exists that infant rats are capable of such simple associative learning (Goldman and Tobach, 1967).

Before closing this discussion on the possible mechanisms that may modulate the course of maternal behaviour, some final comments will be made on the moment-to-moment control of nursing, i.e., what specifically brings the mother to the nest, and what is responsible for the termination of a nursing period.

As far as the initiation of nursing is concerned, one possibility has already been mentioned, that the pups may call the mother to the nest and once the mother is within contact-range, thermotaxis and nipple-seeking occur, and nursing is initiated. Another is that some threshold of engorgement is reached following the progressive filling of the mammary glands during the time when the mother is not nursing, and the mother goes to the litter to relieve this distension, as Cross (1952) and Findlay and Tallal (1971) have suggested for the rabbit, and others (see Lehrman, 1961) for other species. In the rat, several studies indicate that nursing behaviour has no dependency on mammary gland distension or milk secretion (see Munn, 1950; Lehrman, 1961) although others have suggested that the condition of the mammary glands may be involved in the initiation and maintenance of the nursing-suckling relationship (Rosenblatt and Lehrman, 1963). However, if this were the sole motivation to nurse one would expect that with the increases in milk yield through the period of lactation, engorgement would occur more rapidly at later stages and the frequency of nursing should increase. The findings from the present study show rather that the frequency of nest-periods shows a slight decline through lactation, although, as was previously mentioned, actual nursing may not have occurred in all the periods that were included in the analysis. Also against the "mammary gland condition" theory of nursing motivation are the findings that totally mammectomised (Moltz, Geller, and Levin, 1967) or thelectomised mothers (Moltz, Geller, and

Levin, 1967; Moltz, Levin, and Leon, 1969) behave maternally.

Newton and Newton (1967) and Findlay (1971) have suggested a parallel between nursing and coitus in that, for the repeated occurrence of both, they must be a pleasurable experience. Thus, the mother might vicariously present herself to the litter at regular intervals in order to enjoy the stimulation of suckling. If this is the case for the rat, the apparent decrease in visits to the nest, and increases in the inter-nursing interval, would together suggest that some progressive satiation of this desire sets in through lactation. This is uhlikely, as if a mother is presented with a 1-day old litter on her 10th day of lactation, she spends an amount of time with the litter that is appropriate to the age of the litter and not to the stage of her lactation (Grota, 1973).

Neither is there any clear consensus on the underlying mechanism controlling the termination of nursing. It may be, as some have suggested, that prolonged suckling is uncomfortable for the mother and she leaves the nest for this reason. Seitz (1958) suggested that the mother could "fatigue" of nursing. Findlay and Tallal (1971) have extended the analogy between nursing and coitus to suggest that the end of a nursing period may be associated with some event akin to orgasm, but again, if this suggestion holds any generality there is the problem of interpreting the changes in orgasmic behaviour that presumably would arise from being given a younger litter. The answer may be that the reduced vigour of the suckling stimulus from young pups extends the latency to orgasm.

Kumaresan, Anderson, and Turner (1967) have implicated oxytocin in the regulation of the nursing period, having observed that the administration of exogenous hormones increased milk yield but not litter-weight gain; the regular administration of oxytocin, however, did produce significant increases in litter-weight, presumably by allowing the pups to receive more of the available milk (Kumaresan and Turner, 1966). Thus, under normal conditions, nursing may be terminated when the supply of endogenous oxytocin becomes exhausted. The contingent relationship between intermittent ME and oxytocin release (Wakerley and Lincoln, 1971) emphasises an immediate dependency of ME on oxytocin, and MEs may cease when the neurosecretory source of the hormone temporarily expires. This might mean that the mother terminates the nursing period if ME has not occurred for some time, or that the mother somehow becomes "aware" of this depletion in the oxytocin store and, anticipating no further MEs, terminates the nursing period. Both these possibilities would suggest a temporal relationship between the last ME and the end of the nursing period although in the little data pertaining to this aspect of nursing in the Wakerley, Hill, and Lincoln (1973) study, no obvious relationship is apparent. If it were the case that the availability of oxytocin limited the number of MEs and thereby limited the duration of the nursing period, then as the duration of the nursing period decreases through the course of lactation (Grota and Ader, 1969; present study), the availability of oxytocin, and the number of MEs in a

given nursing period, should also decrease (see page 263). Further, the administration of exogenous oxytocin should extend the duration of the nursing period as Kumaresan, Anderson, and Turner (1967) have suggested; unfortunately this measure was not employed in the study in which oxytocin was given (Kumaresan and Turner, 1966).

The suggestion that oxytocin is limiting to the duration of the nursing period, and hence that the availability of oxytocin decreases through lactation, is difficult to reconcile with the observation that mothers at Day 10 of lactation, when given a 1-day old litter, will spend an amount of time with the litter that is appropriate to the age of the litter (Grota, 1973). This would require that the progressive decline in oxytocin availability through to Day 10 was swiftly reversed to accommodate the marked extension of nursing that occurs under these circumstances. This suggests, in effect, that ocytocin is not so directly involved in the regulation of the length of the nursing period, although the findings of Kumaresan and Turner (1966) still require an explanation. Perhaps when extra milk is available following exogenous hormone administration, the attendant mammary engorgement in conjunction with the exogenous oxytocin results in a shortening of the latency to first ME, and an increase in the subsequent frequency of MEs within a particular time (cf. Wakerley, Hill, and Lincoln, 1973), and the amount of milk released at ME may also be increased. Such changes would obviate the need for an explanation on the basis of an increase in the duration of nursing.

One other possibility that has been considered in the course of the present studies is that the control of temperature, this time on the maternal side, is involved. On numerous occasions it was observed that, upon leaving the nest, the mother would roll onto her side and back to expose the nipple-line on the ventral surface. This behaviour is rarely seen in the non-pregnant rat, and it appeared that the lactating rat was attempting to achieve more rapid cooling of the ventrum than would otherwise occur in the normal laying position in which the ventral surface abutts on the floor of the cage. This view was strengthened by the observation that the frequency of assuming this position increased at higher ambient temperatures. In addition, it was observed that upon leaving the nest the mother would also commence grooming, particularly of the ventral surface. Several studies have focused on this self-licking behaviour of the pregnant (Rosenblatt and Lehrman, 1963; Roth and Rosenblatt, 1967; Roth and Rosenblatt, 1968; Steinberg and Bindra, 1962) and lactating rat (Christophersen and Wagman, 1965). The prevention of self-licking through pregnancy by the use of a neck-collar was found to result in a 50% reduction in mammary development, and it was concluded that the self-stimulation of nipple-line licking was involved in mammary gland development (Roth and Rosenblatt, 1968). Christophersen and Wagman (1965) found reduced growth rate in litters reared by mothers who had worn collars during pregnancy, which presumably was a reflection of the inhibition of mammary development.

The demonstrations, however, that the saliva-spreading which accompanies grooming is an important mechanism by which rats may reduce heat through evaporative cooling (Hainsworth, 1967, 1968; Hainsworth and Epstein, 1966; Hainsworth, Stricker, and Epstein, 1968) suggests an additional function of this grooming behaviour. Rosenblatt and Lehrman (1963) and Roth and Rosenblatt (1967) found that although there was little change in the duration of nipple-line grooming between early and mid-pregnancy, there was a substantial increase in this area between mid- and late-pregnancy; in the early postpartum period the licking in this area was almost negligible (Rosenblatt and Lehrman, 1963). It is tempting to attribute the course of such directed licking to the associated changes in surface area to body-volume ratio that occur during this time. The substantial increases in body weight during pregnancy are not compensated for by a proportionate increase in body surface area, and it would be expected that some behavioural adjustment would accompany the increasing difficulty in temperature regulation. The typical reduction in activity that occurs as parturition approaches (Rosenblatt and Lehrman, 1963) would reduce the generation of body heat but it is also possible that evaporative cooling resulting from an increasing amount of grooming would aid further in the dissipation of heat. Thus, the effect of wearing a collar during pregnancy may be to inhibit behavioural adjustment to the tendency towards hyperthermia, and perhaps it is hyperthermia that is responsible for the inhibition

of normal mammary gland development. Benson and Morris (1971) found that mothers exposed daily to 37°C for 7 hours in the last two weeks of pregnancy subsequently showed impaired lactation, reflected in a reduced growth rate of foster young. The immediate decline in licking in the early postpartum period (Rosenblatt and Lehrman, 1963) may therefore be a result of the sudden increase in surface area to body-volume ratio that follows parturition.

Perhaps the obvious function of the ventral surface grooming that follows a nursing period is the cleansing of the nipples and the area around them. Another possibility is that licking, and perhaps saliva spreading are used at this time to facilitate dissipation of heat. It would be useful, therefore, to consider the temperature changes that a mother undergoes during a nursing period. In the interval when the mother has been away from the nest the ectothermic pups will have lost heat down the temperature gradient from about 35° C towards room temperature. When the mother goes onto the nest to nurse, heat will be conducted from the mother to the pups and the temperature of the pups will begin to rise, as heat is retained in a manner different from the situation where the mother is lying on a non-biological medium.

As the temperature of the interface between pups and mother approaches its maximum, this would presumably become uncomfortable for the mother and cause her to terminate the nursing period. That it is the mother and not the pups who is responsible for the termination

of the nursing period is suggested by the observation that pups suckling an anaesthetised mother will continue to do so for hours (Wakerley and Lincoln, 1970) and do not therefore terminate the alliance when satiated. Further, high temperatures, which might be associated with prolonged nursing, do not cause pups to emit distress calls (Allin and Banks, 1971).

If the attainment of a critical temperature during nursing is, in fact, responsible for the termination of nursing by the mother, then as the pups develop homiothermia through the first two weeks, and as the litter gains in mass, the rise time to this critical cut-off temperature would presumably shorten. On this basis, it would be predicted that through lactation the average duration of the nursing period would decrease although no substantial change would be expected in the frequency of nursing. The findings from the present study provide support for this view. Also it would be predicted that, as the ambient temperature increased, the critical temperature would be reached in a shorter time and the duration of nursing should therefore decrease. Such a relationship has been observed in the course of the present investigations. Although the laboratory in which the present study was conducted was thermostatically-regulated, fluctuations in room temperature of as much as 8° F have been recorded, and at the upper limit of variability (about 78° F) a higher proportion of mothers were observed to be out of the nest than at the more usual room temperature (72° F). It will be

recalled too, that in Experiment Va in which there was no control of room temperature, an inverse relationship was found between ambient temperature and the amount of time spent nursing.

The hypothesis of temperature control of nursing may also be used to explain other observations. For example, the characteristic difference in nursing time between the light cycle and the dark cycle (Grota and Ader, 1969; present study) cannot be explained simply on the basis of the fact that the rat is nocturnal and therefore more active at night so that she spends more time away from the nest engaged in other activities; the data from the present study show that the mean nursing frequency over Days 2-14 is virtually identical between dark and light cycles within both the heavy and light groups. Thus, the pattern of nursing behaviour is the same but the duration of nursing periods is less. The shorter nursing period may be explained on the basis that at night the body-temperature is higher than during the day, and therefore the rise-time to the critical temperature would be less.

Further, the findings that mother rats spend more time with a small litter than with a large litter (Seitz, 1954; Grota and Ader, 1969; Grota, 1973) may be due to a combination of two factors assuming that the qualitative features of the nest, in both cases, are comparable (as they are in continuous recording systems): (a) the area of interface between litter and mother, and (b) amount of heat lost by the litter in the between-nursing interval. Thus, in the case of the large litter, (a) would be

greater and the temperature would rise more quickly when the mother is on the nest, and (b) would be less as an increase in the number of pups reduces the surface area to body-volume ratio. Therefore, the temperature of the pups would drop less when the mother is off the nest, and when the mother goes onto the nest the higher starting temperature, coupled with the greater area of interface, would act to reduce the time taken to reach the cut-off point. For the small litter, (a) would be smaller and (b) would be greater and the duration of nursing would therefore increase.

The other finding that was previously mentioned which has proved difficult for other theories on the regulation of nursing, *i.e.*, that a lactating mother of 10 days, if given a litter of 1-day age, will spend an amount of time with the litter that is appropriate to the litter's age and not to her stage of lactation (Grota, 1973; see also Hofer and Wiener, 1971) can also be explained by the present hypothesis. The amount of time the mother spends with the litter is determined by the stage of homoiothermic development of the litter and its mass, both of which determine the rise-time to threshold and therefore the duration of the nursing period.

Although the present temperature hypothesis is consistent with the various observations that have been made on lactating rats, and appears to fit the data of the present study and those that have been reviewed here, it would not be tenable unless it could also be demonstrated

that (i) the rat is sensitive to these labile changes in peripheral temperature, i.e., of the skin surface, and (ii) that behavioural adjustments are made to ameliorate a condition of thermal discomfort. Recent work in the field of behavioural thermoregulation suggests that both conditions may be adequately met.

Weiss and Laties (1961) observed, using an operant paradigm, that rats in the cold would lever-press to obtain heat. That core-temperature changed too slowly to mediate such reinforcement suggested that the behavioural adjustment was being made on the basis of peripheral changes in temperature. Using a thermocouple recording device, implanted under the dorsal skin, it was found that the rats employed the operant to maintain a constant peripheral (subcutaneous) temperature. Lipton, Avery, and Marotto (1970) subsequently demonstrated that, in an operant situation where lever-pressing could be used to escape heat of varying intensities, rats maintained skin-temperature within a very narrow band. At room-temperature, the skin-temperatures associated with response initiation and termination were, at their extremes, no more than 2° C apart, and at the two lowest heat intensity levels (lamps of 100W and 150W) were within 1° C of each other. Several other studies have shown a similar behavioural adjustment to thermal stress under a variety of conditions (Lipton and Marotto, 1969; Epstein and Milestone, 1968; Hardy and Murgatroyd, 1968). Thus, it appears that the rat is able to discriminate relatively small excursions

in peripheral temperature and use learned behaviour to achieve thermoregulation within fairly narrow limits. As the temperature range from the time the mother goes on the nest until the time she gets off is probably in the order of several centigrade degrees, it would be very unlikely if some temperature control were not effected. A schematic summary of this temperature control mechanism is shown in Figure 35. It should be stated in conclusion that such a mechanism is entirely untested as yet, and is hypothesised here to fit the present data and incorporate the observations of others; however, it would appear to be a profitable and interesting line for further investigation.

To summarise this discussion on possibility (iv) that maternal behaviour is modulated by factors in addition to those that are associated with nourishment of the litter: the evidence is conclusive that the mother is responsible for controlling the temperature of the litter over the first 2-3 weeks, and this function is as important as that of providing nourishment for the growing litter. Acoustic signals from the litter may serve, not only to protect against individual isolation, but also to bring the mother to the nest and initiate a nursing period. Although temperature-loss appears to be the principal stimulus for the initiation of these distress calls, other physiological and perhaps learned stimuli, may assume differential importance through the early developmental period. Once the nursing period is under way, the temperature of the litter will begin to rise and may reach a level at which the mother begins

to experience discomfort. She may then disengage herself from the litter to protect against hyperthermia. As the pups develop homoiothermic control, and as the litter increases in mass, the rise-time to this cut-off temperature would be expected to shorten. Increases in maternal body-weight in the first half of lactation will increase the vulnerability of the mother to hyperthermia and therefore should augment the decrease in rise-time.[1] The overall effect of this thermal interaction may be responsible for the decline in the duration of nursing-periods and the decline in total nursing time that has been reported here and elsewhere. Although nursing data was not obtained in the present study beyond the second week, it follows from these observations that the decline in maternal body-weight in the second half of lactation should decrease the mother's tendency towards hyperthermia while nursing, and this, together with a postural change to nursing from a lateral position, should allow sufficient time to maintain a reasonable level of nursing until the time of weaning.

The preceding discussion has been concerned with the possible role of four major sets of variables in the modulation of maternal

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[1] Increases in prolactin levels in the postpartum period (Amenori, Chen, and Meites, 1970) may reduce susceptibility to thermal stress; Thoman, Wetzel, and Levine (1968) found that Nembutal anaesthesia, which depresses body-temperature in the non-pregnant rat, did not achieve this effect in lactating rats. Other hormones may also be involved in dampening the effects of acute temperature fluctuations (see Verniko-Danellis, 1972).

behaviour. Although certain biases may be apparent in the discussion (at least in terms of completeness) the intention has not been to suggest that only one of the four is responsible for the pattern of maternal behaviour that has been described here and in other continuous recording studies. The temperature hypothesis has been reviewed in some detail because it accommodates most of the observations and findings on maternal behaviour and also because it has not previously been formulated. It is very likely that more than one, possibly all four, and perhaps other mechanisms are involved in a complex temporally-interrelated fashion, but without further work a dominant role may not yet be ascribed to any one, or combination of, the variables that have been discussed. Besides appraising some of the many factors that may be involved in the modulation of maternal behaviour, the other purpose of the present discussion has been to provide a background against which the findings of the present study, and those of the study which follows, might be interpreted. The final part of the present discussion is therefore concerned with the differences in nursing behaviour between heavy and light mothers, and the apparent consequences of these different nursing regimes on the development and adult behaviour of their foster litters.

Nursing Behaviour:--The nursing data show that heavy mothers spend less time with their litters than do light mothers. Although a slight difference in frequency of nest-periods is apparent between the two groups, the lower nest-time in the heavy group is achieved also by

a decrease in the duration of nursing periods. Two explanations may be suggested for these differences.

The first relates to the possibility of structural and functional differences in the mammary glands of the two groups of mothers. As was indicated earlier, the differences in body-weight between the two groups at the time of breeding were probably due chiefly to differences in age. As it has been demonstrated that the mammary glands undergo a phase of allometric growth from the prepubertal stage to at least 100 days (Sinha and Tucker, 1966; see also Schmidt, 1970; Cowie and Tindal, 1971) it follows that the heavier and older rats would have had more mammary tissue at the start of pregnancy than the lighter, younger rats. As the amount of tissue growth during pregnancy and lactation is a function of the amount present at the start of pregnancy (see Schmidt, 1970) it follows too that the heavy mothers would have more mammary tissue by parturition, and perhaps through lactation have a higher level of functional activity compared with the light mothers. In support of the above, Pritchard and Tucker (1970) found that, in three groups of rats bred at different ages (44, 63, and 82 days) mammary gland development by the time of parturition, as measured by DNA content, was greater in the older rats. Also, Moon, Griffith and Turner (1959) found that maternal body-weight was highly correlated with dry weight of fat-free mammary tissue, and Hanwell and Linzell (1972) report that mammary gland weight is a constant proportion of body-weight for rats

in full lactation.

It might be argued therefore that enhanced structural and functional mammary gland development in the heavy group of mothers was responsible for the shorter duration of the nursing period, perhaps by allowing a greater amount of milk to pass to the pups in a given time. Thus, when the pups had received a sufficient volume of milk the nursing period was terminated. However, such an explanation would require that pup satiety was involved in the regulation of the length of the nursing period, and other evidence suggest that this is not so. Pups put to an anaesthetised mother will continue to suckle for hours (Wakerley and Lincoln, 1970). It may indeed by the case that the heavy mother passes a greater amount of milk to her pups in a given time, but there is no evidence to suggest that the duration of nursing is controlled directly by the amount of milk that has passed from mother to pup. In fact, if the heavy mothers did produce more milk it might even be expected that the nursing period would have been prolonged.

Another possibility to consider is that the increases in mammary gland development by parturition, that are presumed to have occurred in the heavy group, may not have been retained in the postpartum period. Pritchard and Tucker (1970) assayed their three groups of mothers on the 16th day of lactation and found no significant differences in mammary gland DNA content at this stage. Neither were there any significant differences in litter-weight gain between Days 3-16 although the data, as

for mammary gland DNA, show a trend towards higher values in the older group of mothers. To summarise: the evidence from other studies suggests that the heavier and older mothers would have enjoyed a greater degree of mammary development by parturition, although if this advantage were retained in the postpartum period it would be unlikely that a concomitant increase in milk production would have acted to reduce the duration of the nursing period.

The second explanation involves the temperature control mechanism discussed earlier. The greater body-weight of the heavy mothers would result in a decrease in the ratio of surface area to body-volume, and therefore heat-dissipation would be less efficient in these mothers. The increased mass of these mothers may have also resulted in a more effective interface between the ventral surface of the mother and the litter especially in the nest-boxes used in this study. Both these factors would act to reduce the rise-time to the cut-off level at which maternal hyperthermia is threatened and would therefore shorten the duration of the nursing period. The slight increases in body-weight of the heavy-reared pups over the light-reared pups would act to further reduce the duration of nursing. The results of Experiment Va and Vb in which a similar relationship was indicated between maternal body-weight and nursing time in the normal nest, indicate that the findings of the present study are not simply an artifact of the nest-box design.

Observations on Development

(a) Morphological Development:--The significant increases in body-weight of the heavy-reared litters over the light-reared litters may have been due to a higher production of milk by the heavy mother although Grosvenor and Turner (1959) report that litter-growth is not affected by increases in milk-supply up to a point. While it may seem incongruous that litters who spent less time with their mothers gained more weight, it is clear that there is little overall relationship between nest-time and weight gain. Despite a substantial decline in nest-time through the first two weeks of lactation litters maintain a steady growth-rate. The presumed increase in milk-production in the heavy mothers would be expected to increase the degree of mammary engorgement between nesting periods, and this, in turn, may change the latency to first ME, the amount of milk released at ME, and the frequency of MEs (Wakerley, Hill, and Lincoln, 1973). Thus, the heavy mother may be capable of passing a greater amount of milk to her litter in a shorter time.

Although the weight gain of the litter is frequently used as an index of lactational performance of the mother (see Kon and Cowie, 1961), Kumaresan, Anderson, and Tucker (1967) and others have questioned this assumption. The enhanced growth rate of heavy-reared pups may not therefore be a simple manifestation of superior lactation by the heavy mother. Tactile and thermal stimuli may also be involved in the regulation of growth as Sayler and Salmon (1969) have suggested,

and the different nursing regimes of heavy and light mothers might certainly be associated with an altered thermal and tactile stimulus input to the litter. The shorter nursing period of the heavy mother would produce an altered pattern of temperature change in the litter both during the nursing period and in the time when the mother is absent from the nest. An altered pattern of tactile stimulation both from litter-mates and from the mother may result and might be augmented by the differences in nursing frequency between the groups. Such a conclusion is supported by the findings of a study by Hofer and Grabie (1971) in which it was demonstrated that specific changes in pup activity, and hence the amount of between-pup tactile stimulation, occurred in conjunction with different phases of the nursing cycle.

The apparent relationship described here between the prenatal : postnatal litter-size ratio and pup weight-gain adds support to the proposal above that prenatally-established differences in mammary development are preserved, with functional consequences, in the postpartum period. Although this is the first indication in the rat that prenatal mammary gland development may be regulated by the size of the litter that the mother is carrying, the phenomenon has previously been considered by others (see Blaxter, 1961) and has been demonstrated in mice (Bateman, 1954; Machin and Page, 1973). The present results, although based on a limited number of observations, also suggest an interaction of this effect with maternal body-weight. The effect of maternal body-weight upon

litter-weight gain appears to be mitigated by increases in the litter-size ratio. The interaction could be explained simply on the basis of structural and functional differences in the mammary glands, or may instead be due to a more complex interaction between mammary gland differences associated with the litter-size ratio and the different stimulus input arising from the nursing regime that is associated with maternal body-weight. These preliminary findings warrant further investigation as such factors may be an important source of influence in early growth and development.

No significant differences were found in the other parameters of physical development that were investigated.

(b) Reflex development:--The emergence of the two reflexes studied, particularly that of the startle reflex, showed surprisingly little variability compared with the results of other studies (cf. Ray and Hochhauser, 1969; Schapiro, 1971). This may be due to the precise dating of pup age, the balanced sex-ratio and litter-size, and the uniformity of rearing conditions in the present study. Nevertheless, significant differences between the two groups were detected which could be attributed to the influence of the foster mother.

Although the litters reared by light mothers cannot in any way be considered as "starved," the differences in body-weight indicate that they may have been on a slightly lower plane of nutrition than the heavy-reared pups. In this sense, the finding of a slightly delayed

appearance of reflexes associated with slightly lower weight-gain, may be a subtle manifestation of the relationship that has previously been described between early undernutrition and reflex development (Eayrs and Lishman, 1955; Simonson, Sherwin, Anilane, Yu, and Chow, 1969; Smart and Dobbing, 1971).

The finding that reflex development was advanced in the females of both groups is interesting in view of the prevailing assumption that no such differences exist (see Eayrs, 1951). The detection of sex-differences in the present study may, again, be due to the unusually constant rearing conditions and the balanced sex ratio. In the study by Earys (1951) which was conducted under normal laboratory conditions, with apparently no culling or standardisation of the sex-ratio, it was reported that male pups scored higher than females in the free-fall righting reflex at 15 days of age, and also showed an earlier appearance of the placing reflex. No sex differences were found for the startle reflex or for negative geotaxis (see also Gregory, 1970). In a recent study (Slob, Snow, and de Natris-Mathot, 1973) untreated males showed a slightly earlier appearance of the startle, and free-fall righting reflexes compared with untreated females although the difference was not statistically evaluated.

It is possible, of course, that the stabilised rearing conditions of the present study may have suppressed, or even reversed, sex-differences that would be present in the natural rearing conditions. A more

parsimonious explanation is that the rigid control of postnatal litter and nest parameters that was achieved in the present study optimised the likelihood of detecting these small differences between the sexes. The failure to detect such differences in other studies that have examined the early ontogeny of reflex development under various experimental conditions is probably not a serious one, as normally it would be expected that the distribution of sexes between various groups would be even. However, the hypothesis that androgen secretion differentially favours reflex development in the male (Eayrs, 1951) is difficult to accept in the light of the present findings and those of Experiment VIII.

Observations in the Adult Stage

(a) Female assay:-- The complete "catch-up" in female body-weight at 56 days, coupled with the tendency for brain parameters to be negatively correlated with preweaning growth-rate, appears to be somewhat paradoxical. For example, in studies that have been concerned with the experimental manipulation of growth in infancy (e.g., Lat, Widdowson, and McCance, 1960) it has usually been found that decrements in early growth usually persist well into adulthood. with females showing more "catch-up" in soft tissue than males (Williams, Tanner, and Hughes, 1974). Early undernutrition has also been demonstrated to result in the suppression of brain parameters in adulthood (Winick and Noble, 1966b; Dobbing and Sands, 1971).

The results of these studies are not necessarily in conflict with

the present findings. Although the light-reared females were possibly less well nourished than the heavy-reared females, the pattern of growth and development was well within the bounds of "normality." In fact, the tendency towards higher values of brain parameters in the light-reared group may belie the tacit notion of a continuum between sub-normal and maximal growth, as well as the idea that maximal is somehow optimal. If the structural development of the brain, attained by the adult stage, is taken to reflex the efficacy of early development, the present data suggest rather that optimal may lie somewhere between subnormal and maximal.

The difficulty in describing what is "normal" and what is "optimal" has been discussed by others (Dunn, Murphy, and Rockland, 1946; Blaxter, 1961). Although the treatment of the problem under conditions that are far removed from the natural habitat and therefore from the forces of natural selection may have rendered the issue somewhat academic, some recent considerations may at least suggest a re-examination of the belief that accelerated growth in early life is necessarily beneficial.

Osborne and Mendel (1926) originally suggested that the developmental process may be disrupted with adverse physiological consequences if early growth were unduly rapid. Recent evidence suggests that relatively mild influences in early development, although producing what initially appears to be a suppression of growth, actually result in an enhancement of the structural development of the brain. It has

frequently been demonstrated, for example, that animals which are handled in infancy, or subjected to treatments involving an "enrichment" of stimulus input to the organism, demonstrate changes in adult behaviour that are commonly considered to be more adaptive or "intelligent" (see review by Meyers, 1971).

Altman, Das, and Anderson (1968) handled rats from Days 2-11 and injected labelled thymidine to examine the course of brain cell-proliferation in the post-handling period. Whereas at 11 and 14 days the unhandled animals had heavier brains than the handled animals, at 41 and 101 days there were no significant differences between the two groups. In a pilot study, using animals derived from two litters, these same authors found that, for a similar pattern of brain growth, the rate of cell-proliferation between 11-41 days was substantially higher in the handled group. It was suggested that the apparent deceleration of brain maturation in the handled group allowed for a delay in the proliferation and migration of microneurons, and that this extension of the period of structural plasticity of the developing brain could lead to a more adaptive organisation of the brain by environmental influences. Such "improved" organisation of the developing brain would account for the superior performance of handled animals in problem-solving tasks.

With regard to the present findings on the female brain in adulthood, it appears that the early lower growth rate in the light-reared litters had no adverse effects on later body-weight or on structural

parameters of the brain, rather it appears that some slight advantage may have been gained.

(b) Open-field behaviour:--Perhaps the most interesting findings in the present experiments were the correlative relationships between male behaviour in the open-field, and the amount of time spent with the mother in the first two weeks of postnatal life. The inverse relationship between the two most frequently measured parameters of open-field behaviour, activity and defecation, is a common finding in open-field studies. Activity was positively correlated, and defecation negatively correlated, with nest-time. These results are of particular interest when compared with those of Seitz (1954) who found that mothers rearing small litters tended to be more "maternal" than those rearing large litters, and that in adulthood animals from small litters were more active and less emotional than those from large litters. The findings on maternal behaviour were not anticipated by Seitz, as, on the basis of a simple reinforcement paradigm, it might have been expected that differences between mothers would have gone in the other direction, i.e., that more maternal behaviour should have occurred where there were more reinforcers (pups). That the converse was the case suggested that maternal responsiveness might undergo "fatigue" when over-stimulated by a large litter. Since these original observations, several studies have confirmed the inverse relationship between maternal behaviour and litter-size, using a continuous recording system (Grota

and Ader, 1969; Ader and Grota, 1970; Grota, 1973). The notion that "fatigue" is involved in the control of maternal behaviour is unlikely as a mother at Day 10 of lactation will swiftly increase her nest-time if given a younger-litter (Grota, 1973). An explanation on the basis of thermal factors seems more likely.

The effects of early experience on adult behaviour were interpreted by Seitz (1954) as indicative of a relationship between early "competition and frustration" and the later response to a novel situation. Animals from large litters would have experienced more competition and frustration in their food-seeking behaviour in infancy, and, in adulthood, subsequently showed a greater "expectancy of danger" in the novel situation by being less active and more emotional. On the basis of Denenberg's hypothesis (1964), however, the extra stimulation arising from the competition within the litter should have decreased the level of emotionality in adulthood.

In the present study, with the litter-size variable held constant, the same sort of relationship obtains between the behaviour of the mother and the adult behaviour of the animals, i.e., where the mother spent less time with the litter, more emotionality and less activity was observed in adulthood. It has already been posited here that the different nursing patterns of heavy and light mothers may have been responsible for the early developmental differences, and while the possibility remains that competition and frustration levels may have been

different in the two groups of litters and may, therefore, have been responsible for the effects observed, it seems that maternal behaviour may be implicated more directly than this, and may indeed be the critical variable.

Schaefer (1963; 1968a; 1968b) has proposed, as an alternative to the stimulation hypotheses (Levine, 1962b; Denenberg, 1964), that the temperature variable is critically involved in the mediation of effects arising from early handling. This view has generally received support from others (Hutchings, 1963; 1965; 1967; 1968; Caldwell and Kesner, 1966; McIver, Jeffrey, Stevenson, and Nielson, 1968) and changes in maternal behaviour attendant with such treatment have also been implicated (Young, 1965; Caldwell and Kesner, 1966). The findings of the present study, in conjunction with the theoretical model that has been proposed for the temperature control of nursing, can be used to link together these two theoretical standpoints of stimulus input and temperature change.

For the heavy mothers, the shorter duration of nursing periods coupled with the longer between-nursing intervals would presumably be associated with a different cyclic pattern of temperature change in the litter compared with the light group. As adherents to the temperature hypothesis have noted, even slight changes in temperature may alter the kinetics of metabolic events in early infancy, and may hold important consequences for development and adult behaviour. The difficulty,

however, is that these cyclic changes in litter temperature are probably also associated with cyclic changes in activity and other physiological parameters. These, in turn, may change the degree of within-litter stimulation and hence the level of stimulus-input during infancy. It is difficult to conceive of an experimental design that would effectively separate these two classes of variables as they are so inextricably related to each other. Despite this difficulty, the present findings offer additional information pertinent to the theoretical issues surrounding the early handling phenomenon, and emphasise the critical role of the mother in the mediation of the effects.

To conclude the present discussion, it would perhaps be worthwhile to briefly consider the significance of the behavioural changes that have been observed here to be correlated with preweaning experience. The behaviour of the light-reared males in the open-field, if considered more adaptive and appropriate, could be viewed as corroboration of the female assay data where it appeared that a slower pattern of early growth and development might have been more "optimal" than the higher growth rate and slightly precocious reflex development of the heavy-reared animals. This might suggest that what has been observed in the present study is a slight shift in the degree of "infantilisation" (Altman, Das, and Anderson, 1968) or neoteny (see Ashley-Montagu, 1955).

The principle of neoteny generally holds that the longer an organism spends in its early developmental stages the more beneficial it is,

in that, during this plastic stage of ontogeny the organism is subject to environmental influences that may mold structural and functional development in a fashion that will allow the organism to respond more appropriately to the contingencies of the environment in adulthood. In essence, it is the zeitgeist of the epigenetic approach. As Schapiro noted,

> "...a compressed temporal-experiential interval may prematurely foreclose behavioural adaptability. In fact, an optimum interval may be necessary for brain development and experience consolidation. During this time afferent input is constructing a neuroanatomical foundation for later behaviour."
>
> Brain Development and Behaviour, 1971, p. 327.

The finding that the period of postnatal neurogenesis is extended following early handling (Altman, Das, and Anderson, 1968) appears to be an example of experimentally-induced neoteny, and is of particular interest in view of the critical role in brain function that has been ascribed to these late-appearing microneurons (Scheibel and Scheibel, 1964; Altman, 1967).

Clearly, there is some lower limit to the rate at which early development may proceed and still hold "beneficial" consequences for the organism. Where development is restricted by nutritional deficiency, or where other influences prevent the realisation of at least "normal" development, the consequences may prove to be chronically detrimental to the organism. What is needed is a pattern of early development that optimises the interaction between the genetic substrate and environmental

influences such that the organism is best adapted to its particular ecological niche.

In this regard, it cannot with any certainty be stated what the characteristics of "intelligent" or adaptive behaviour are with respect to the present findings. Intuitively, it might be felt that lowered emotionality and a higher level ot exploratory behaviour are a combination that indicate good adaptiveness to a novel situation. On the other hand, it might be considered that to experience fear and to minimise exploration are a more effective means of dealing with new circumstances. Thus, while the light-reared males may indeed have been neotenised through the pattern of their mother's nursing behaviour, it may not necessarily be concluded that their behaviour in adulthood reflected such an advantage. This general problem in the assessment of behaviour will be discussed again in the study which follows.

SUMMARY

The findings and observations of the present study, in which natural differences in maternal body-weight were studied, may be summarised as follows:

(i) Body-weight of female rats at time of breeding had no apparent effect on gestation period, litter size, or birth-weight of offspring.

(ii) Time of parturition was related to the photoperiod and was determined, within very narrow limits, by litter-size.

(iii) Birth-weight of offspring was inversely related to litter-size.

(iv) Following pregnancy and lactation, primiparous light mothers incurred a higher overall weight gain by the post-weaning stage than did heavy mothers.

(v) Heavy mothers spent less time throughout lactation with their litters compared with light mothers. The difference was achieved apparently through a reduction in the duration of the nursing period and by a slight reduction in the frequency of nursing period.

(vi) Body-weight gain of the litter was determined, in part, by the body-weight of the foster mother and by the prenatal:postnatal litter size ratio of the foster mother. These two variables appeared to interact.

(vii) The development of reflexes in pups was influenced by body-weight of the foster mother.

(viii) Brain parameter values of the female offspring, in adulthood, appeared to show an inverse relationship with early growth and development.

(ix) Behaviour of the male offspring in the open field, in adulthood, was highly correlated with preweaning experience.

The modulation of maternal behaviour during the course of lactation

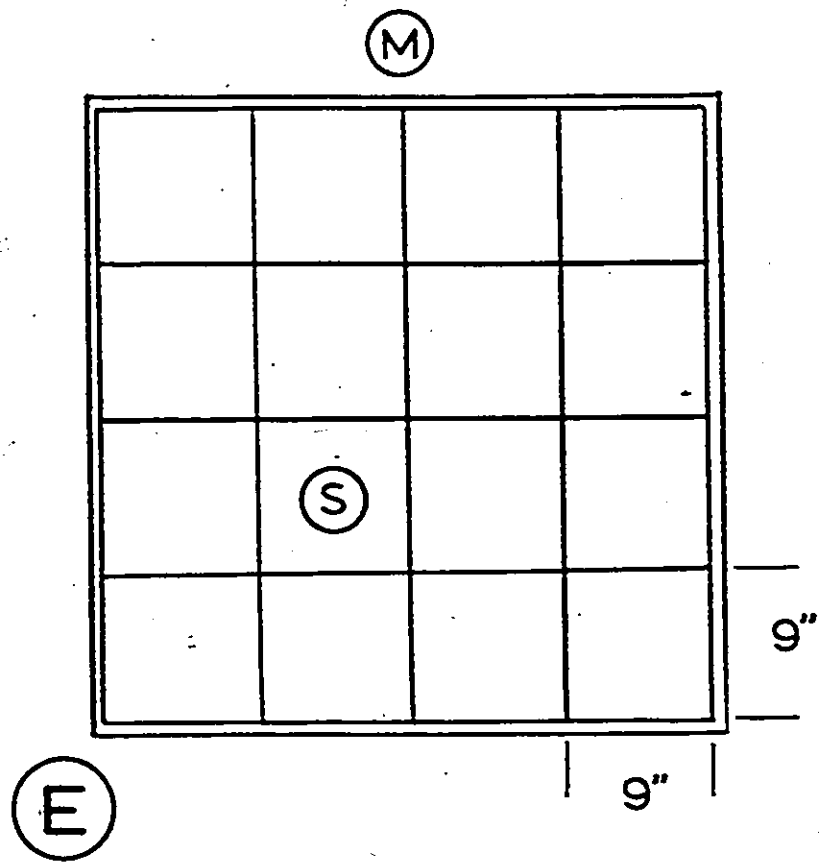
was discussed and a theory based on temperature dependence was proposed. Differences in early development and adulthood were interpreted from the theoretical standpoints of early handling and neoteny.

Figure 21

Plan of open-field and summary of observations. M = mirror, S = starting square, and E = experimenter.

Figure 21

OPEN-FIELD PLAN



OBSERVATIONS

1. Activity: recorded as the number of lines crossed in the 5 minute observation period. The criterion for a line-cross was met when the subject placed both forefeet over the line.

2. Defecation: measured as the number of fecal boluses deposited in the 5 minute period.

3. Close-Rear: recorded when the subject reared on the hind-feet and touched one of the side-walls with one or both fore-feet. These rears always occurred in one of the 12 perimeter squares.

4. Open-Rear: recorded when the subject reared on the hind-feet and did not touch the side-wall. These rears usually occurred when the subject was oriented away from the side-walls.

Figure 22

Group mean maternal body-weights through pregnancy and lactation for the eight light mothers (open circles) and either heavy mothers (circles). At each stage the two curves are significantly different from each other (see text for details).

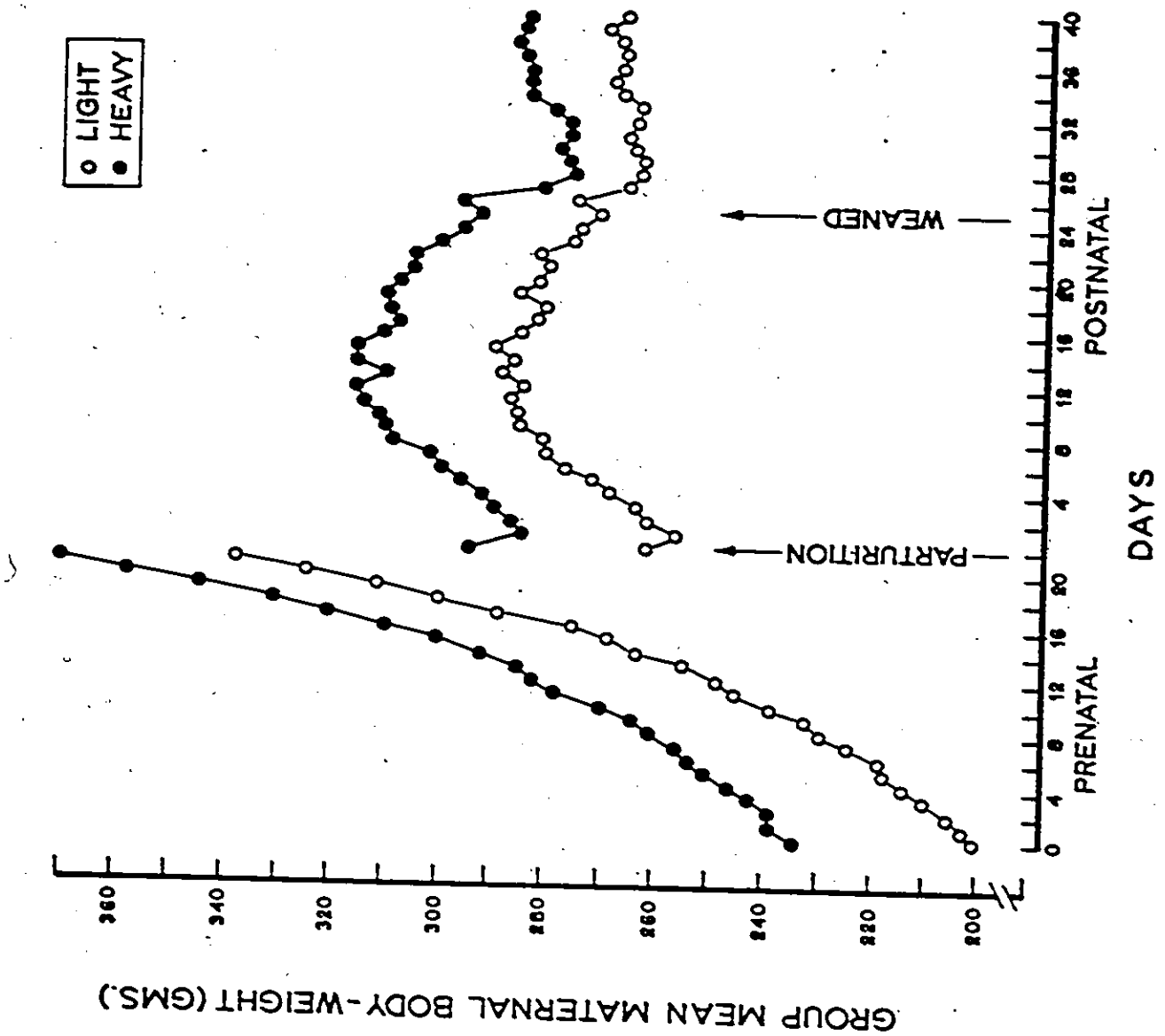

LIGHT
HEAVY
WEANED
PARTURITION
PRENATAL
POSTNATAL
DAYS
GROUP MEAN MATERNAL BODY-WEIGHT (GMS.)

Figure 22

Figure 23

Group mean daily nest-time in late pregnancy and over first fifteen days postpartum for five heavy mothers (circles) and six light mothers (open circles). Standard error of the mean indicated by vertical bars.

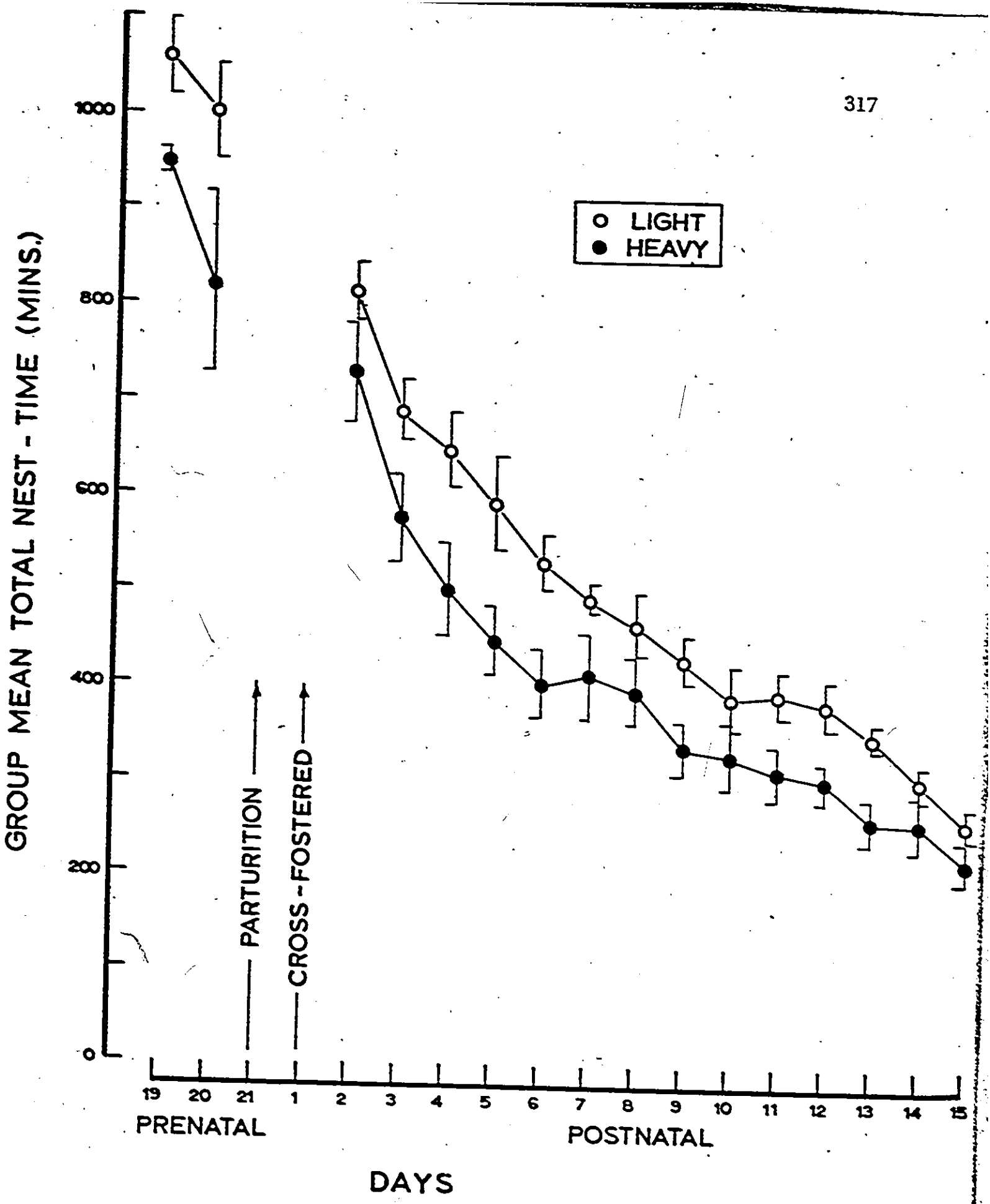

GROUP MEAN TOTAL NEST - TIME (MINS.)
100
80
60
40
20
0
LIGHT
HEAVY
PARTURITION
CROSS-FOSTERED
19
20
21
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
PRENATAL
POSTNATAL
DAYS

Figure 23

Figure 24

Group mean frequency of nest-periods for six light mothers (open circles) and five heavy mothers (filled circles) in light period (upper record) and dark period (lower record), in late prenatal period and first two postnatal weeks.

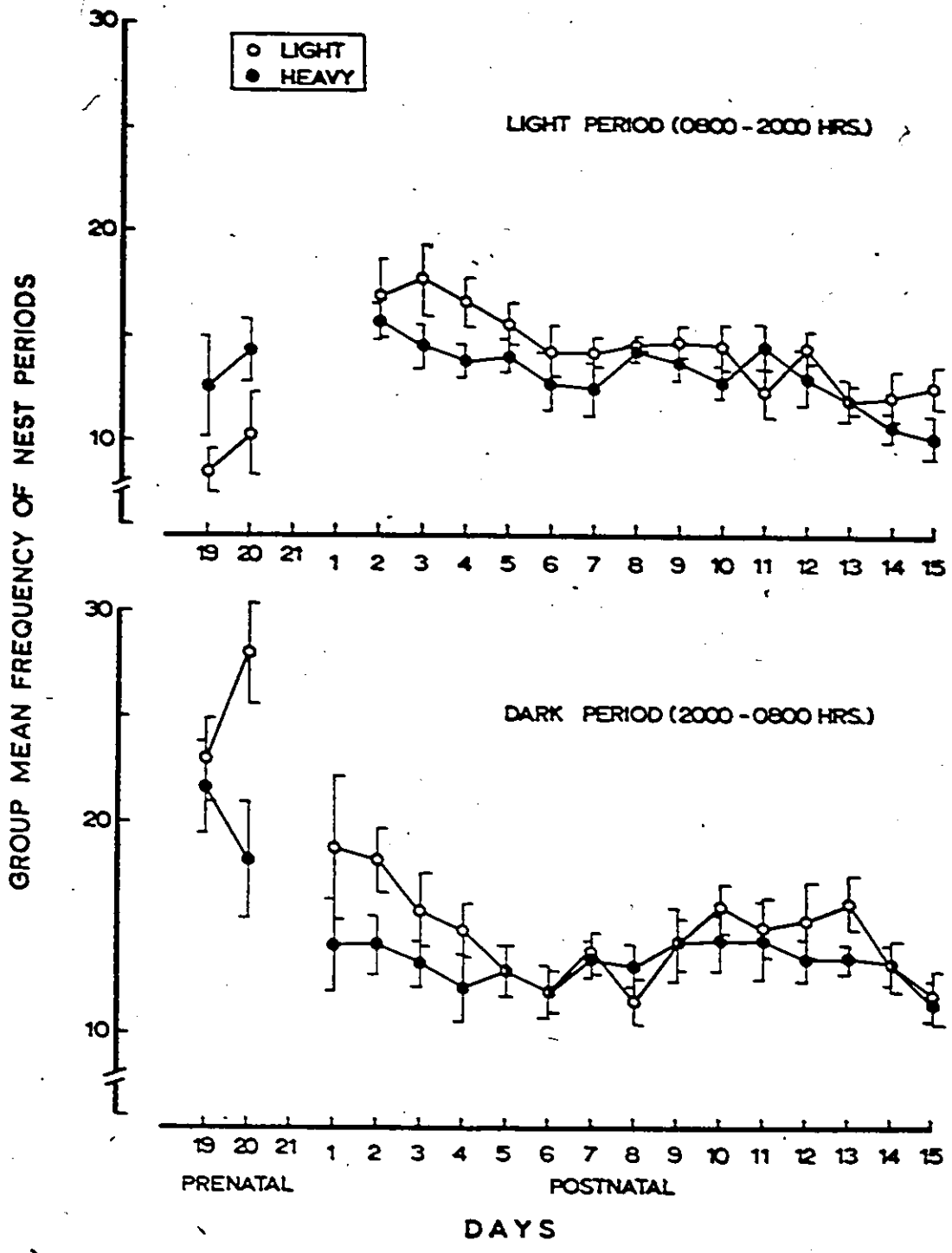

LIGHT
HEAVY
LIGHT PERIOD (0800 - 2000 HRS.)
DARK PERIOD (2000 - 0800 HRS.)
GROUP MEAN FREQUENCY OF NEST PERIODS
30
20
10
19 20 21 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
PRENATAL
POSTNATAL
DAYS

Figure 24

Figure 25

Group mean frequency of nest-periods in late prenatal period and first two postnatal weeks.

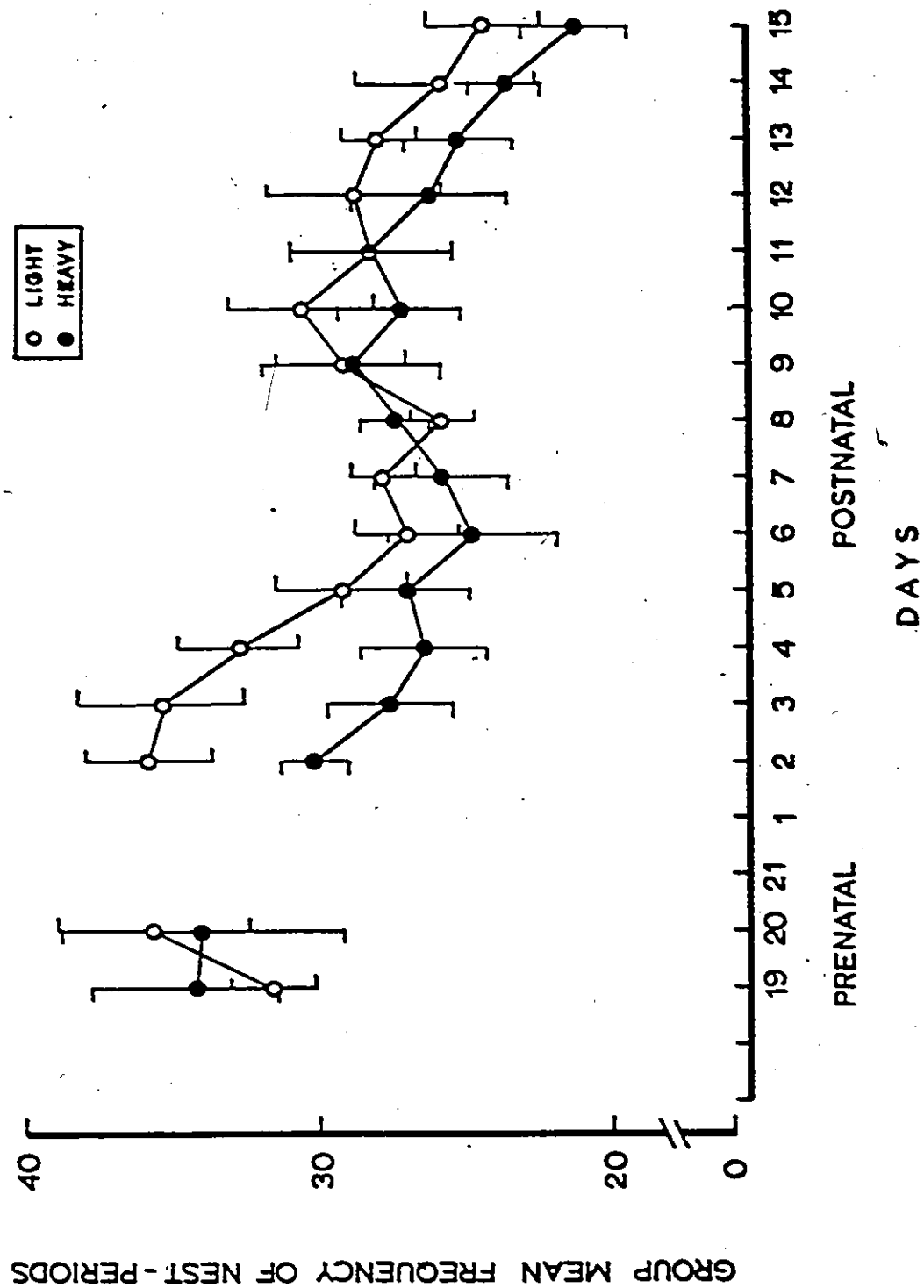

LIGHT
HEAVY
GROUP MEAN FREQUENCY OF NEST-PERIODS
40
30
20
0
19 20 21 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
PRENATAL
POSTNATAL
DAYS

Figure 25

Figure 26

Frequency distributions of nest-periods for the six light mothers on prenatal Day 20 and Postnatal Days 2, 8, and 14. Light period (0800-2000 hr.) shown on left and dark period (2000-0800 hr.) on right.

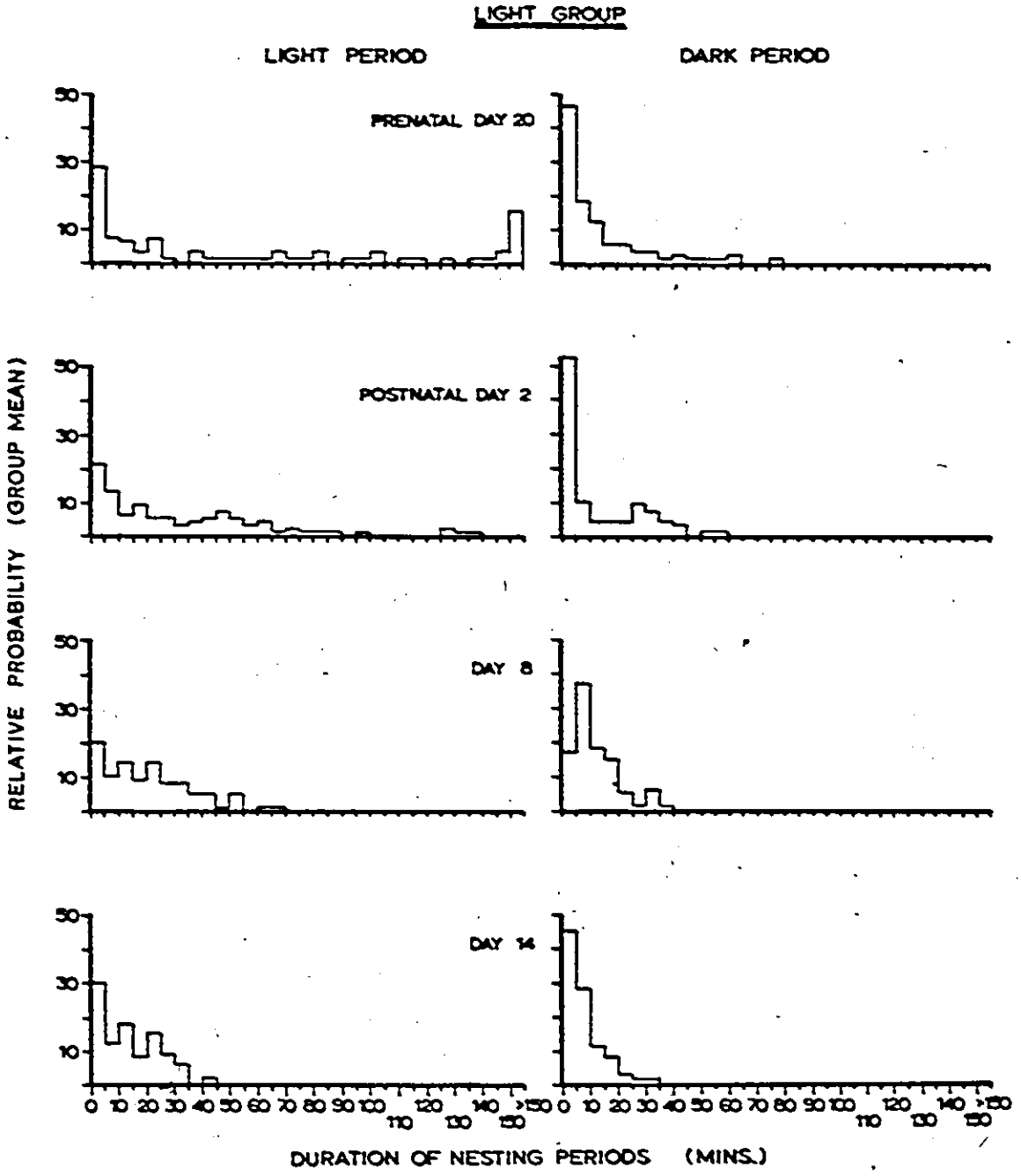

LIGHT GROUP
LIGHT PERIOD
DARK PERIOD
PRENATAL DAY 20
POSTNATAL DAY 2
DAY 8
DAY 14
RELATIVE PROBABILITY (GROUP MEAN)
50
30
10
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 >150
DURATION OF NESTING PERIODS (MINS.)

Figure 26

Figure 27

Frequency distributions of nest-periods for the five heavy mothers on prenatal Day 20 and Postnatal Days 2, 8, and 14. Light period (0800-2000 hr.) shown on left and dark period (2000-0800 hr.) on right.

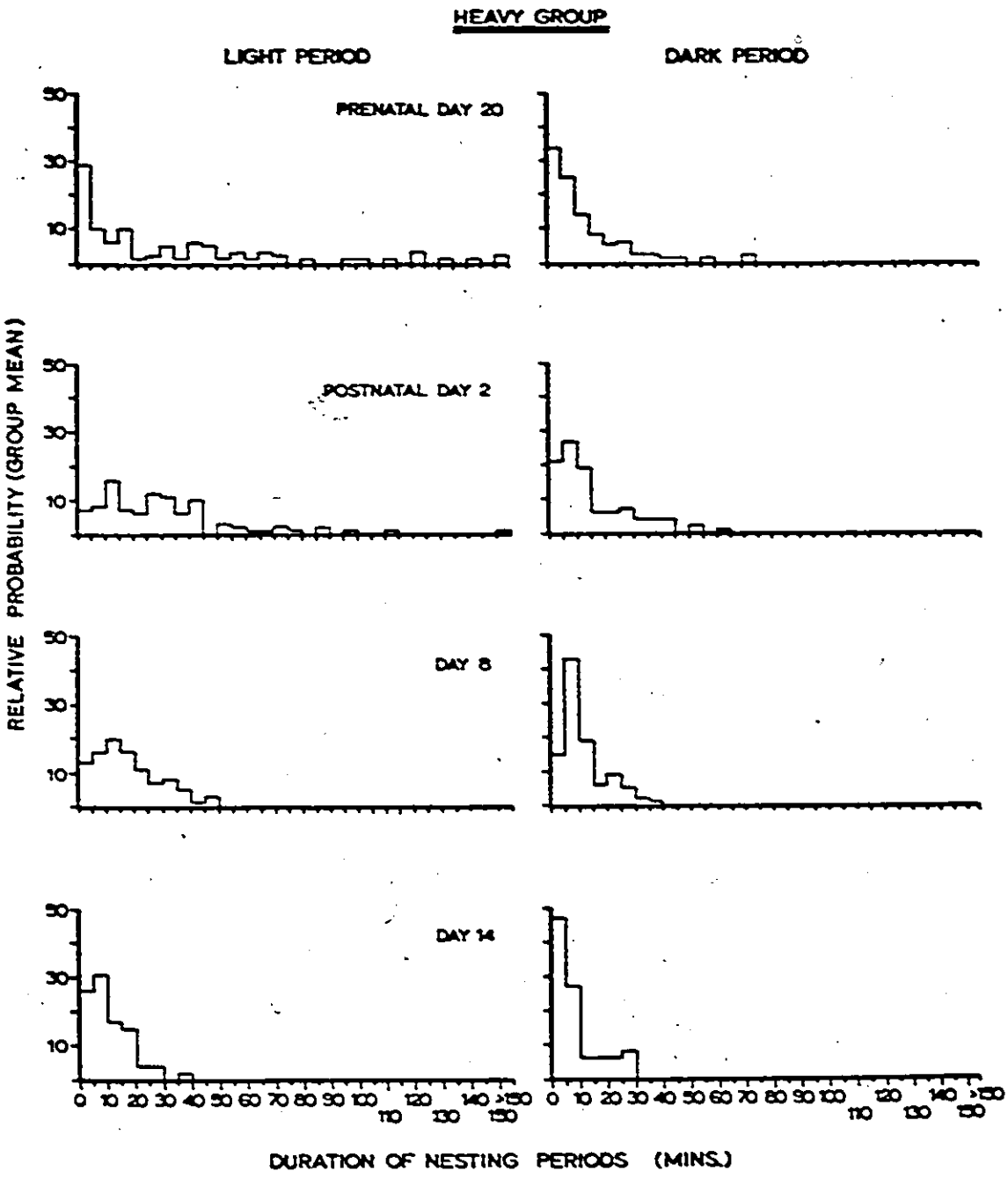

HEAVY GROUP
LIGHT PERIOD
DARK PERIOD
PRENATAL DAY 20
POSTNATAL DAY 2
DAY 8
DAY 14
RELATIVE PROBABILITY (GROUP MEAN)
50
30
10
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 >150
DURATION OF NESTING PERIODS (MINS.)

Figure 27

Figure 28

Group mean duration of nest-periods obtained by dividing daily nest-time (see Figure 23) by daily frequency (Figure 25). Light group represented by open circles, heavy group filled circles, and standard error by vertical bars.

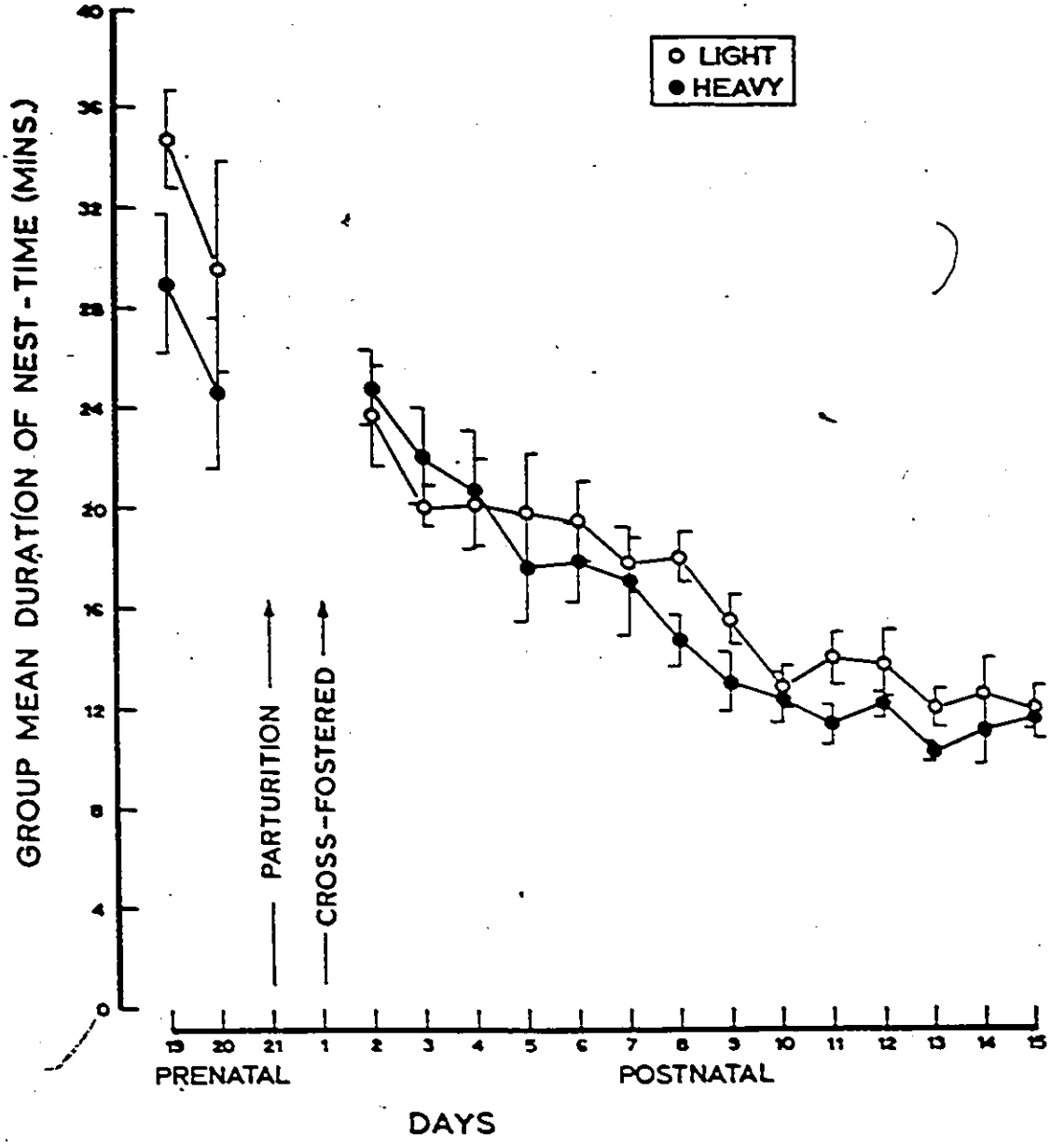

LIGHT
HEAVY
GROUP MEAN DURATION OF NEST-TIME (MINS.)
PARTURITION
CROSS-FOSTERED
PRENATAL
POSTNATAL
DAYS

Figure 28

Figure 29

Frequency distributions of off-nest periods for the six light mothers on postnatal Days 2, 8, and 14.

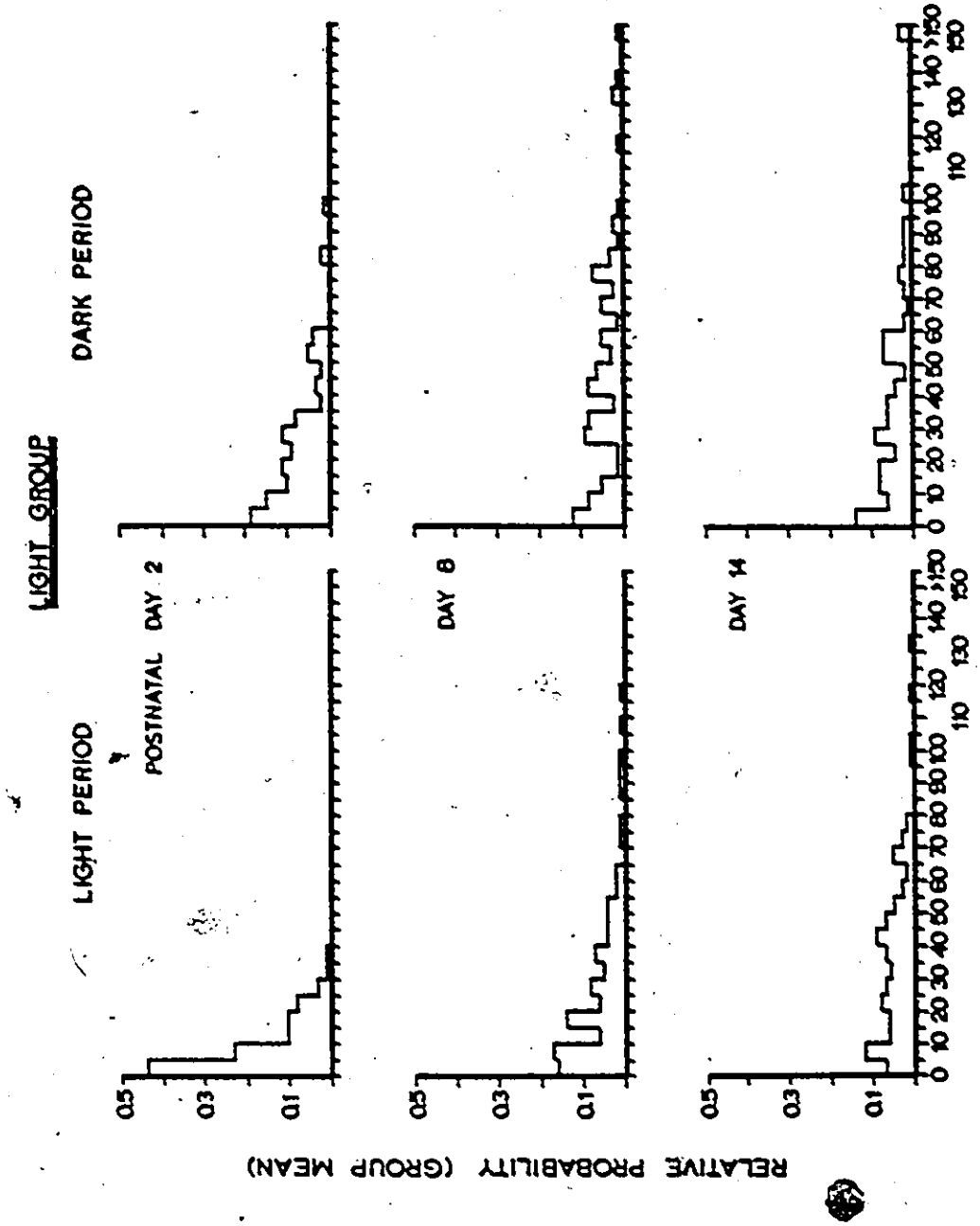

LIGHT GROUP
LIGHT PERIOD
DARK PERIOD
POSTNATAL DAY 2
DAY 8
DAY 14
RELATIVE PROBABILITY (GROUP MEAN)
DURATION OF OFF-NEST PERIODS (MINS.)

Figure 29

Figure 30

Frequency distributions of off-nest periods for the five heavy mothers on postnatal Days 2, 8, and 14.

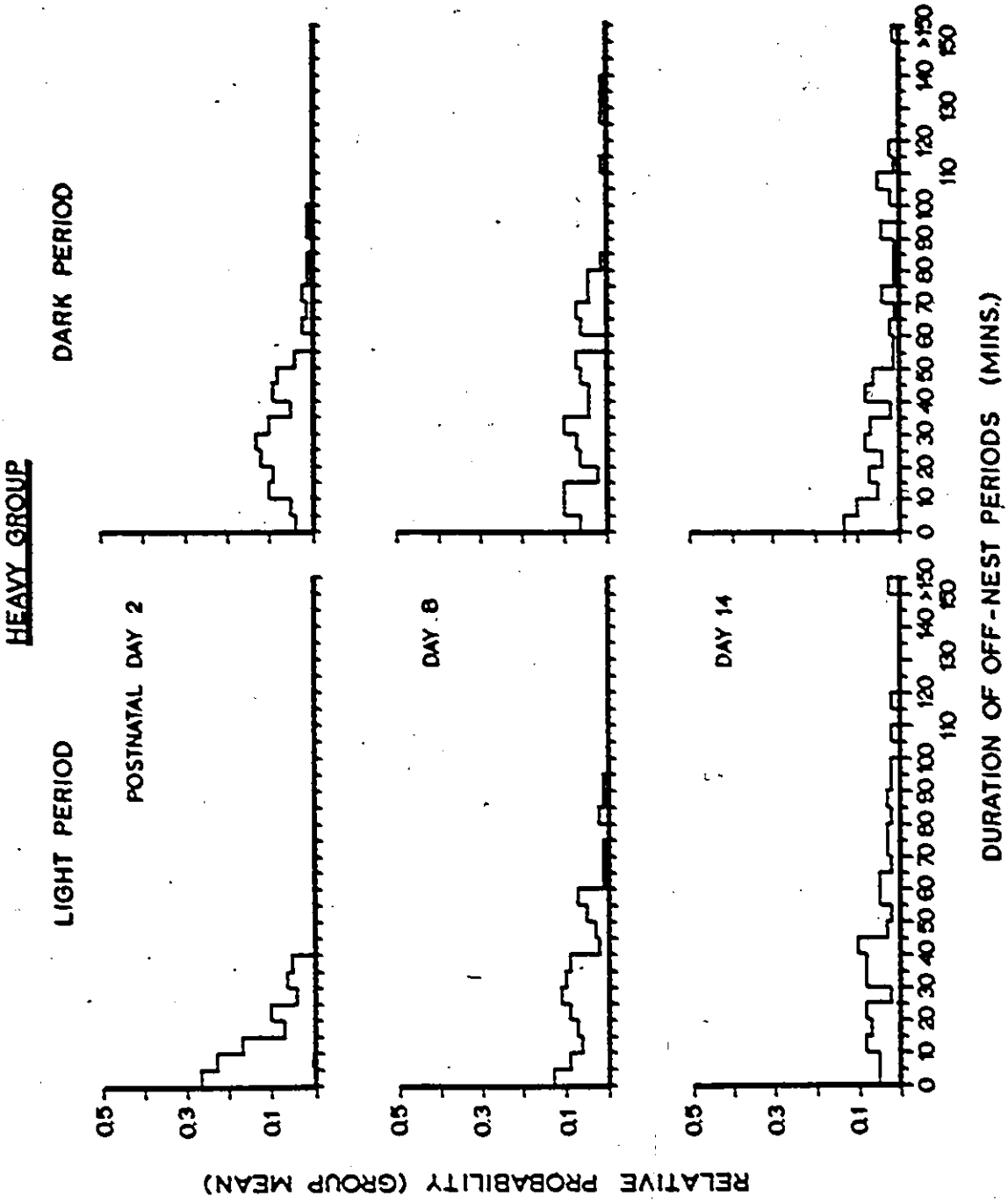

HEAVY GROUP
LIGHT PERIOD
DARK PERIOD
POSTNATAL DAY 2
DAY 8
DAY 14
RELATIVE PROBABILITY (GROUP MEAN)
0.5
0.3
0.1
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 >150
DURATION OF OFF-NEST PERIODS (MINS.)

Figure 30

Figure 31

Mean weights of litters reared by light mothers (open circles) and by heavy mothers (filled circles). Vertical bars indicate standard error of the mean.

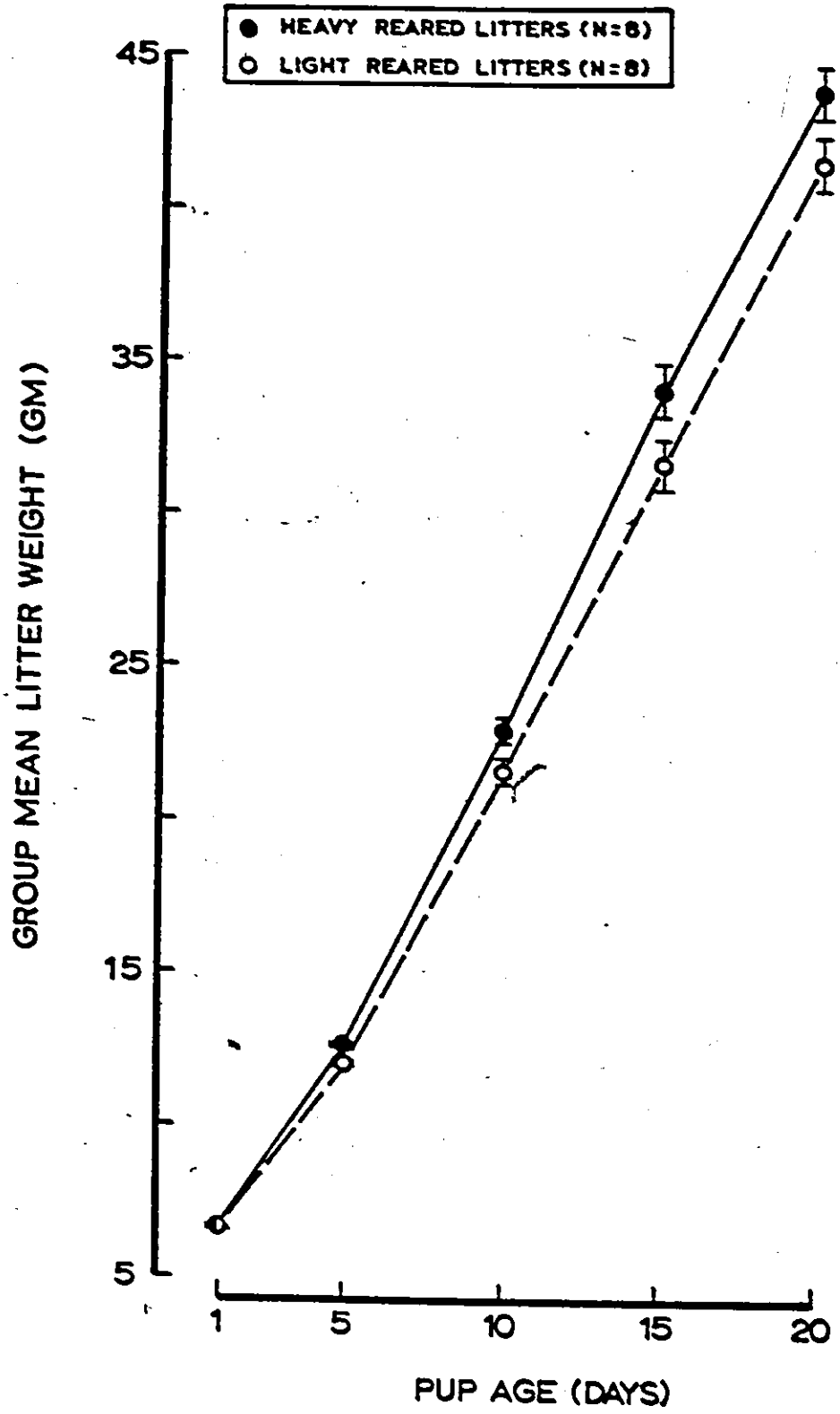

HEAVY REARED LITTERS (N=8)
LIGHT REARED LITTERS (N=8)
GROUP MEAN LITTER WEIGHT (GM)
45
35
25
15
5
1
5
10
15
20
PUP AGE (DAYS)

Figure 31

Figure 32

Regression of litter weight gain on maternal body-weight for each of five prenatal to postnatal litter-size ratios.

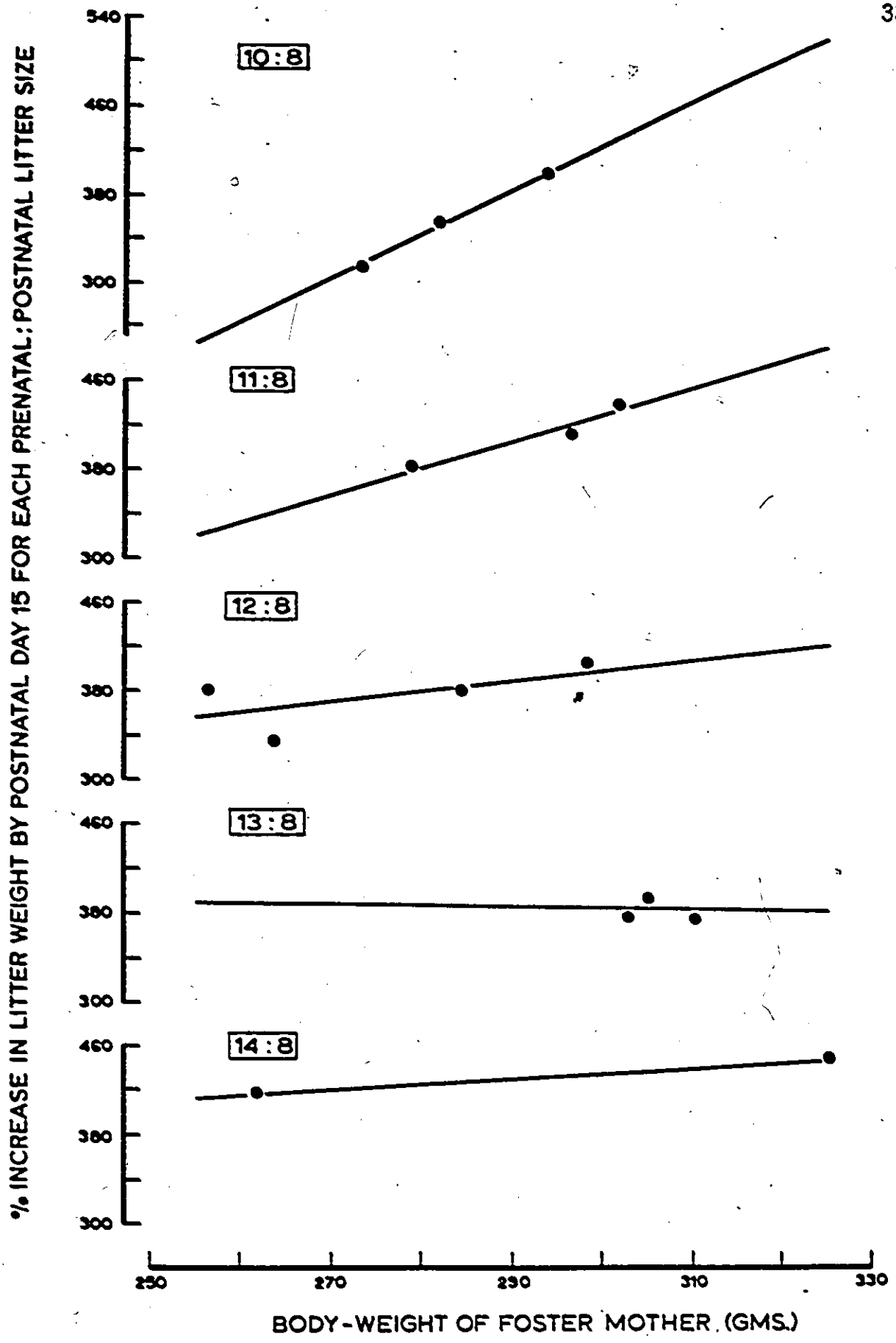

% INCREASE IN LITTER WEIGHT BY POSTNATAL DAY 15 FOR EACH PRENATAL: POSTNATAL LITTER SIZE
10:8
11:8
12:8
13:8
14:8
540
460
380
300
250
270
290
310
330
BODY-WEIGHT OF FOSTER MOTHER (GMS.)

Figure 32

Figure 33

Developmental parameters analysed by sex irrespective of prenatal or postnatal conditions. Females: open circles; males: filled circles.

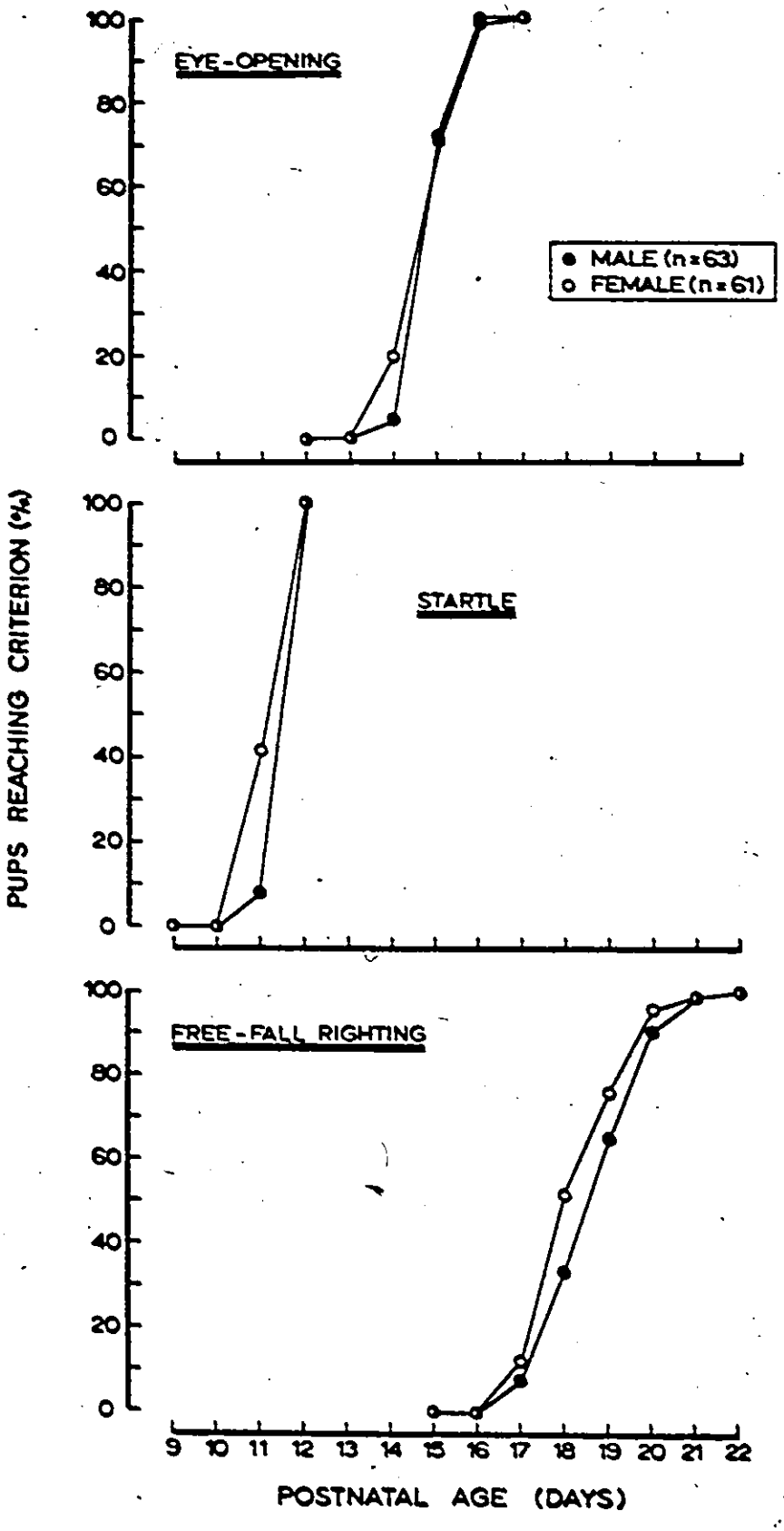

EYE-OPENING
MALE (n=63)
FEMALE (n=61)
STARTLE
FREE-FALL Rrighting
PUPS REACHING CRITERION (%)
POSTNATAL AGE (DAYS)
0
20
40
60
80
100
9 10 11 12 13 14 15 16 17 18 19 20 21 22

Figure 33

Figure 34

Activity and defecation of males from eight heavy-reared, and eight light-reared litters over five days of testing in adulthood.

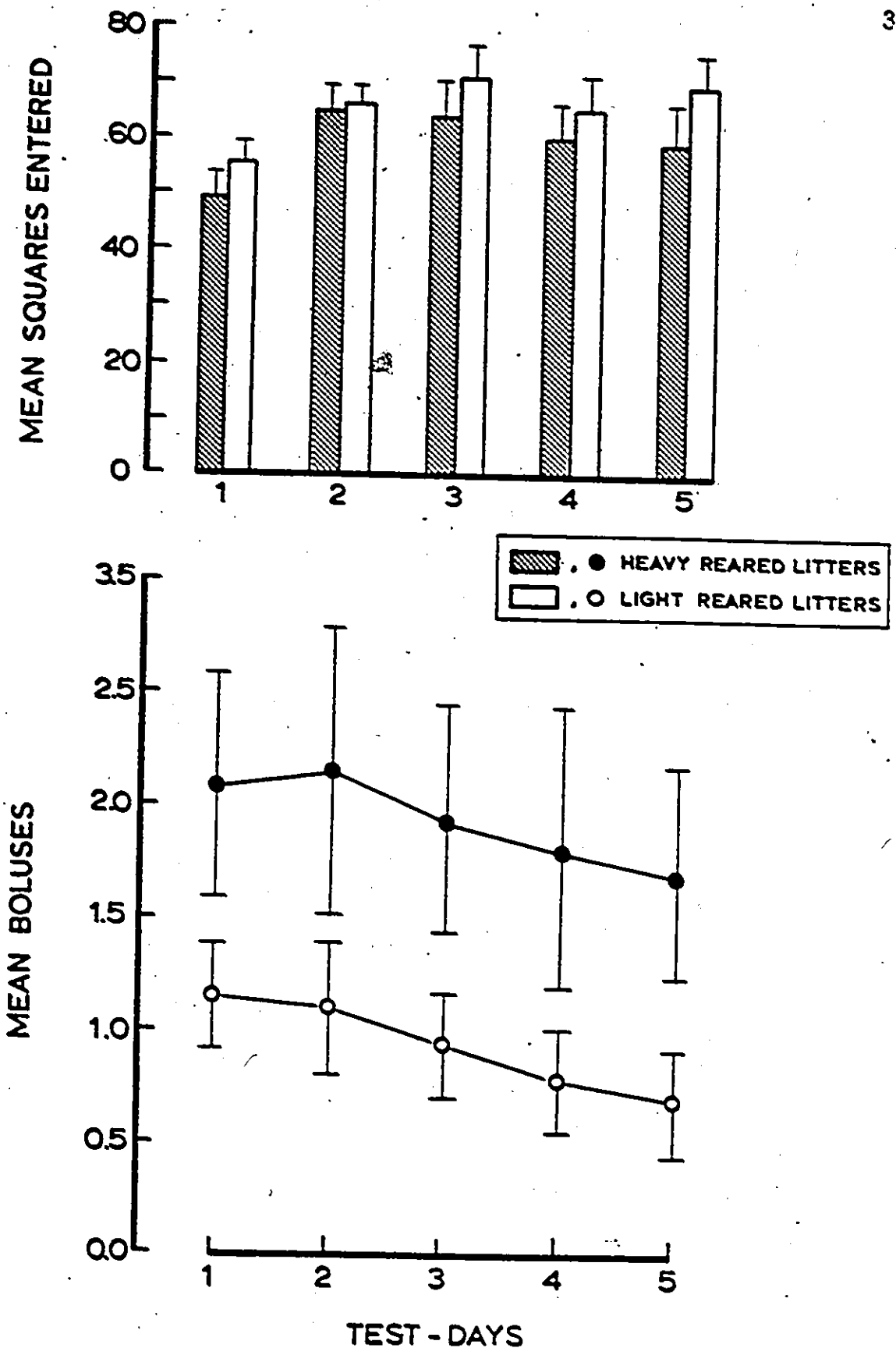

MEAN SQUARES ENTERED
80
60
40
20
0
1
2
3
4
5
HEAVY REARED LITTERS
LIGHT REARED LITTERS
MEAN BOLUSES
3.5
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
5
TEST - DAYS

Figure 34

Figure 35

Highly schematised model for temperature control of nursing behaviour. Cut-off level is shown constant at about 37° C through lactation, although this may shift at various stages due to dampening effects of hormones and other factors (see text). In saw-tooth plot, vertical arrows indicate when mother gets on the nest; broken line shows increase in pup-temperature concomitant with increase in temperature of maternal ventrum. Mother gets off nest at cut-off level and solid line shows decline in pup temperature which follows. Arrows indicate direction of temperature change. Average duration of daily nursing indicated by solid bars on abscissa (Days 1-15 postpartum).

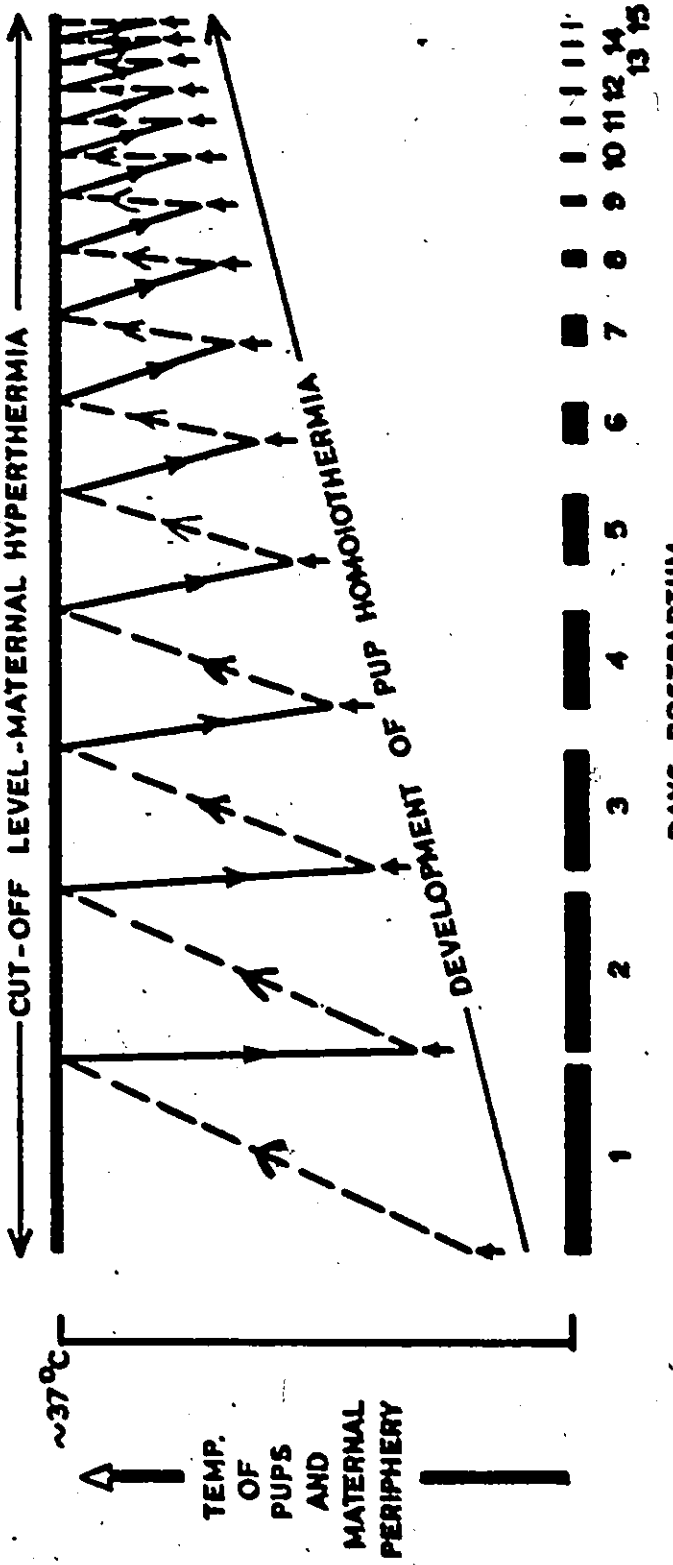

CUT-OFF LEVEL-MATERNAL HYPERTHERMIA
DEVELOPMENT OF PUP HOMOIOTHERMIA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS POSTPARTUM
~37°C
TEMP. OF PUPS AND MATERNAL PERIPHERY

Figure 35

Table XXII

Experimental design. For each of the four postnatal groups, the first letter denotes the prenatal origin of the mother and the second letter the prenatal origin of the pup. H = heavy; L = light.

Table XXII

Postnatal Group	Prenatal Origin		Number of Litters
	Mother	Pup	
H/H	H	H	4
H/L	H	L	4
L/L	L	L	4
L/H	L	H	4

Table XXIV

Group mean (±standard error) nest-time in light period (0800-2000 hr.) and dark period (2000-0800 hr.) for five heavy and six light mothers.

Table XXIV

	GROUP MEAN NEST-TIME ± S.E. (min.)					
	HEAVY MOTHERS			LIGHT MOTHERS		
	0800–2000	2000–0800	Ratio	0800–2000	2000–0800	Ratio
Prenatal Day 19	639 ± 8	310 ± 17	2.06	668 ± 10	391 ± 35	1.71
20	575 ± 28	245 ± 70	2.35	652 ± 18	353 ± 35	1.85
21	-	-		-	-	
Postnatal Day 1	-	487 ± 72		-	398 ± 44	
2	509 ± 44	220 ± 25	2.31	560 ± 22	253 ± 17	2.21
3	373 ± 32	202 ± 35	1.84	448 ± 31	240 ± 10	1.87
4	312 ± 26	188 ± 28	1.66	413 ± 31	233 ± 16	1.77
5	269 ± 22	177 ± 17	1.52	375 ± 33	216 ± 23	1.74
6	241 ± 20	161 ± 21	1.49	321 ± 26	208 ± 22	1.54
7	241 ± 22	170 ± 27	1.41	311 ± 21	179 ± 15	1.74
8	248 ± 20	145 ± 17	1.71	313 ± 26	151 ± 12	2.07
9	216 ± 43	121 ± 16	1.78	281 ± 17	165 ± 7	1.70
10	187 ± 15	143 ± 20	1.31	237 ± 22	153 ± 16	1.55
11	184 ± 5	126 ± 25	1.46	237 ± 21	157 ± 9	1.51
12	170 ± 14	131 ± 17	1.30	225 ± 21	155 ± 8	1.45
13	145 ± 14	116 ± 10	1.25	206 ± 9	143 ± 12	1.44
14	137 ± 11	121 ± 19	1.13	192 ± 17	110 ± 14	1.75
15	124 ± 14	94 ± 9	1.31	155 ± 10	102 ± 16	1.51
TOTAL (Days 2–15)	3356	2115		4274	2465	

Table XXV

Group mean (+standard error) frequency of nest-periods in light period (0800-2000 hr.) and dark period (2000-0800 hr.) for five heavy and six light mothers.

Table XXV

	GROUP MEAN FREQUENCY ± S.E.			
	HEAVY MOTHERS		LIGHT MOTHERS	
	0800–2000 hr.	2000–0800 hr.	0800–2000 hr.	2000–0800 hr.
Prenatal Day 19	12.60 ± 2.34	21.60 ± 2.20	8.57 ± 1.13	23.0 ± 1.95
20	14.33 ± 1.58	18.20 ± 2.91	10.29 ± 1.73	28.0 ± 2.35
21	-	-	-	-
Postnatal Day 1	-	14.20 ± 2.27	-	18.83 ± 3.38
2	15.67 ± 0.20	14.17 ± 1.40	16.86 ± 1.71	18.29 ± 1.58
3	14.50 ± 0.92	13.33 ± 1.12	17.70 ± 1.60	15.86 ± 1.74
4	13.83 ± 0.87	12.17 ± 1.47	16.71 ± 1.04	14.86 ± 1.34
5	14.00 ± 0.82	13.00 ± 1.15	15.57 ± 1.00	13.00 ± 1.25
6	12.83 ± 1.35	12.00 ± 1.12	14.29 ± 1.21	11.86 ± 0.91
7	12.50 ± 1.31	13.50 ± 0.76	14.14 ± 0.88	13.86 ± 0.74
8	14.17 ± 0.40	13.17 ± 0.95	14.71 ± 0.42	11.43 ± 1.09
9	13.83 ± 0.91	14.50 ± 1.63	14.71 ± 0.61	14.29 ± 1.38
10	12.80 ± 0.66	14.33 ± 1.61	14.43 ± 1.15	16.00 ± 1.13
11	14.50 ± 1.09	14.33 ± 1.91	12.33 ± 1.20	15.00 ± 1.41
12	13.00 ± 1.29	13.50 ± 0.99	14.50 ± 0.67	15.29 ± 1.85
13	12.00 ± 1.06	13.50 ± 0.89	12.00 ± 0.71	10.14 ± 1.39
14	10.67 ± 0.71	13.33 ± 0.88	12.14 ± 1.44	13.29 ± 1.51
15	10.17 ± 0.87	11.33 ± 0.80	12.57 ± 0.97	11.71 ± 1.29
Total (Days 2-15)	184.47	186.16	202.66	200.88
Mean (Days 2-15)	13.18	13.30	14.48	14.35

Table XXVI

Modal frequency of nest-period duration in light and dark periods for heavy and light groups.

Table XXVI

Modal Frequency of Nest-Period Duration

	0800-2000 hr.		2000-0800 hr.	
	HEAVY	LIGHT	HEAVY	LIGHT
Prenatal Day 19	5	5	5	5
20	5	5	5	5
21	5	5	5	5
Postnatal Day 1	5	5	25	5
2	15	5	10	5
3	5	5	10	5
4	20	5	15	5
5	10	5	10	5
6	20	5	5	10
7	15	5	10	10
8	15	5	10	10
9	5	5	5	5
10	10	20	5	5
11	5	20	5	5
12	10	5	10	5
13	5	5	5	5
14	10	5	5	5
15	20	5	5	5

5: duration is 1 - 5 minutes
10: duration is 6 - 10 minutes
15: duration is 11 - 15 minutes
20: duration is 16 - 20 minutes

Table XXVII

Female body-weight and brain assay at 56 days.

Table XXVII

Female Assay at 56 Days

	GROUP MEAN (± Standard Error)			
	H/H	H/L	L/H	L/L
Body-weight (gm.)	188.29 ± 5.03	189.13 ± 9.48	188.88 ± 2.97	188.44 ± 4.65
Brain-weight (mg.)	1.768 ± 0.019	1.779 ± 0.011	1.795 ± 0.026	1.84 ± 0.040
Brain DNA (µg.)	2481.3 ± 74.1	2393.3 ± 36.1	2448.5 ± 29.2	2569.0 ± 37.6
Brain Protein (µg.)	1509.7 ± 63.0	1638.7 ± 94.1	1651.6 ±105.6	1853.7 ± 9.7

Table XXVIII

Measures of open-field behaviour of males in adulthood.

Table XXVIII

Open Field Behaviour of Males Between 77-81 Days

	GROUP MEAN (± Standard Error)			
	H/H	H/L	L/H	L/L
Squares	67.38 ± 8.08	51.65 ± 6.59	60.2 ± 2.40	71.13 ± 8.19
Boluses	1.78 ± 0.71	2.11 ± 0.78	0.99 ± 0.14	0.88 ± 0.33
Close-Rear	19.48 ± 2.30	18.30 ± 0.30	16.10 ± 1.72	19.89 ± 1.34
Open-Rear	7.85 ± 1.66	9.41 ± 2.10	10.90 ± 2.01	10.78 ± 1.64
Body-weight at 90 days	406.1 ± 12.3	418.9 ± 4.2	395.6 ± 15.6	425.7 ± 11.2

Experiment VIII

THE INFLUENCE OF PRENATALLY-ADMINISTERED GROWTH HORMONE ON GESTATION PERIOD, MATERNAL BEHAVIOUR, AND POSTNATAL DEVELOPMENT OF THE OFFSPRING

The results of experiments reported in Section I demonstrated that the administration of growth hormone to the gravid rat produced no augmentation of body-weight or of brain parameters of fetuses assayed near term. The absence of any such changes in prenatal development suggested that previously reported differences in early postnatal development (Clendinnen and Eayrs, 1961; Ray and Hochhauser, 1969) and in adult behaviour (Clendinnen and Eayrs, 1961; Block and Essman, 1965; Ray and Hochhauser, 1969), of the offspring from growth hormone-treated mothers, were not due to a direct action of the hormone on fetal development.

An alternative possibility was raised that the hormone regime may instead produce changes in the mother which might alter the interaction between the mother and her litter during the postpartum period, and be responsible for the expression of developmental and adult differences in the offspring. The studies by other workers, referred to above, do not preclude such an interpretation as all offspring were reared by their natural mothers.

The possibility that the growth hormone-treated mother may affect the development of the pups was suggested by the demonstration in three experiments (II, III, and IV) of a reliable and significant increase in maternal body-weight during pregnancy as a result of the hormone treatment. This increase over that which normally occurs during pregnancy was clearly not due to weight-increases in the conceptus, but the experiments provided no positive evidence of its origin or whether such prenatally-established increases in maternal body-weight persisted into the postnatal period.

The results of other studies (Va, Vb, VII) provided evidence of a relation between maternal body-weight and maternal behaviour in the postpartum period, and in one study (VII) other relations were found between these parameters and early development and adult behaviour of the fostered offspring.

In the final experiment to be reported here, several questions were asked. First, does the administration of growth hormone affect the period of gestation or parturition? Second, do the prenatal increases in maternal body-weight, arising from the growth hormone treatment, persist in the postpartum period? Third, if the body-weight differential with control mothers was maintained during lactation, were there any associated changes in maternal behaviour, or any other changes in the mother consequent to the prenatal hormone treatment? Finally, it seemed important to study the development and

adult behaviour of offspring from the hormone-treated mothers using a cross-fostering regime that would allow the partitioning of possible prenatal and postnatal sources of influence.

Two studies will be reported. In the first (Experiment VIIIa), the effects of two preparations of growth hormone were investigated. Due to anomalous results that seemed to be unrelated to the experimental treatments themselves (see below) the planned postnatal investigations, outlined above, were not made. In the second (Experiment VIIIb), one preparation of growth hormone was used, and as all groups appeared to be normal observations were continued as planned in the postnatal period.

METHOD

Subjects, and the conditions of mating and housing, were as described in Experiment VII.

On Day 7 of pregnancy, subjects were randomly assigned to one of two groups. The growth hormone (GH) groups received daily subcutaneous injections of 3 I.U. of growth hormone contained in 0.2 ml. of physiological saline at pH 8.0 - 9.0 over Days 7-20 (inclusive). In Experiment VIIIa one group of GH subjects received an ovine preparation (NIH-GH-S10) and the other a bovine preparation (NIH-GH-B16). In Experiment VIIIb, all GH subjects received a bovine preparation NIH-GH-B17). The control subjects (CS) in both experiments received the same volume of the vehicle in the same pH range and by the same route. Subjects in all groups were injected and weighed around midday.

On Day 18 of pregnancy, subjects were transferred from the clear plastic cages to the continuous recording cages described previously.

The time of onset of parturition was determined either by direct observation or by estimation from the event record and the state of the litter when first noticed (see Experiment VI). In Experiment VIIIb, a culling and cross-fostering procedure, similar to that employed in Experiment VII, was carried out on postconception Day 22 to provide four groups (Table XXIX) containing four mothers and litters in each

group. However, as seven of the eight GH mothers gave birth on Day 22 (see Results) it was necessary to foster pups from GH mothers to CS mothers who had given birth the previous day (Group CS/GH), and to foster CS pups born on Day 21 to GH mothers giving birth on Day 22 (Group GH/CS). Thus, in some cases one-day old pups were moved "backwards" to a mother who had just given birth, and in other cases newly-born pups were moved "forwards" to mothers who had already been lactating for one day. In groups CS/CS and GH/GH, the temporal synchrony between pup age and maternal stage of lactation was not disturbed by the fostering procedure.

Postnatal observations on these sixteen mothers and their litters were the same as for Experiment VII with the exception that litter-weights were recorded daily up to Day 20. Litters were weaned on Day 25. Maternal body-weight was recorded daily up to Day 40 at which time mothers were sacrificed and body-weight, body-length, and organ weights were recorded.

At 56 days, female offspring were sacrificed and brain assays performed. Between 75-80 days males were tested in the open-field. The observations were as described in Experiment VII with the following modification. Instead of being placed directly in the open-field at the start of a trial, each subject was first placed in a closed rectangular box affixed at floor level to one of the side-walls of the open-field. After 10 sec. had elapsed, a door was raised which permitted entry

directly into the field. The latency for the subject to enter the field was recorded, and if the subject did not venture into the field within two minutes a maximum latency score of 120 sec. was given and the subject placed manually in the field. The door was closed immediately to prevent subjects passing from the field back into the waiting box. The five-minute daily trial commenced as soon as the subject entered the field voluntarily or was placed there by the experimenter.

RESULTS

Experiment VIIIa.

Of five subjects receiving the ovine growth hormone preparation two showed a plateau in body-weight around Days 17-18 and did not deliver their litters. The remaining three had not delivered their litters by Day 23 by which time two were bleeding from the vagina. They were sacrificed and, at autopsy, massive haemorrhages were apparent in the uterus. Fetuses appeared to be normally developed and alive in one mother and dead in the other. The remaining mother had not delivered by Day 24 and her fetuses were found to be dead at autopsy.

Four GH subjects receiving the bovine preparation delivered their litters in the early part of the light period on Day 22. Mean gestation time was 535.25 ± 0.75 hr. One mother gave birth to dead fetuses on Day 23 (not included in estimate of gestation time).

Of the CS subjects run concurrently with the above two groups, six delivered their litters in the light period of the 21st day and one delivered overnight between the 20th and 21st day. Mean gestation time was 514.85 ± 2.05 hr. which was significantly less than that of the bovine GH group ($p < 0.001$).

During pregnancy, signs of respiratory infection were apparent

in both GH and CS groups and the incidence of resorption was abnormally high. Following the observations reported above, no further measures were made and the study was discontinued. The laboratory was cleared of all animals and after two weeks Experiment VIIIb was commenced using a new stock of animals.

Experiment VIIIb

Of nine GH subjects receiving the bovine preparation one delivered in the light period of Day 21 and the remaining eight just before or in the light period of Day 22. Mean gestation time was 533.02 ± 2.75 hr., and mean litter size 10.56 ± 0.73. Body-weight of male pups at birth was significantly greater than that of females ($p < 0.05$, paired comparisons t-test). A significant negative regression of birth-weight (Y) on litter-size (X) was found for the eight litters born on Day 22 ($Y = 8.060 - 0.144X$; p(zero slope error)< 0.05). No relationship was found between gestation time and litter-size for those litters born on Day 22.

Of twenty-one control mothers, nineteen gave birth in the light period of Day 21 and two in the light period of Day 22. Mean gestation time was 518.01 ± 1.36 hr. which was significantly less than that of the GH group ($p < 0.0001$).[1] Mean litter size was 11.00 ± 0.51 which

[1]For comparison purposes, Figure 36 shows the distribution of birth-times for control mothers from Experiment VII (26) and Experiment VIII (21) and mothers treated with NIH-GH-B17 in Experiment VIII (9).

was not significantly different from the GH group ($p > 0.05$). Body-weight of male pups at birth was significantly greater than that of females ($p < 0.01$, paired comparisons t-test). A significant negative regression of birth-weight (Y) on litter-size (X) was found for the 19 litters born on Day 21 ($Y = 6.933 - 0.124X$; p (zero slope error) < 0.001). The regression of litter-size (X) on gestation time (Y) was negative ($Y = 517.779 - 0.156X$) but not significant (p (zero slope error) > 0.05). Mean birth-weights of pups born to the two CS mothers on Day 22 were not significantly different from those of GH pups born on Day 22 ($p > 0.05$).

Maternal Body-weight:--Prenatal and postnatal body-weight curves for the sixteen mothers that were studied postnatally are shown in Figure 37. After two injections of the hormone, the eight GH mothers showed a significantly greater increase in body-weight than the eight CS mothers (Day 9: $p < 0.01$). By day 21 there was a mean difference of 50 gm. between the two groups which was highly significant ($p < 0.00001$). In the postpartum period, the body-weight curves of the two groups were significantly different from each other at every stage, although by the time of weaning the difference had closed to 40 gm. On Day 40 the mean difference had closed still further to 22 gm. although the GH group were still significantly heavier than the CS group ($p < 0.005$).

Multiple comparisons were made among the four groups of mothers to confirm that the two GH groups were significantly heavier than the two CS groups over Days 2-15 postpartum (timed from conception or from

parturition). In both cases, the two GH groups (GH/GH and GH/CS) were found to be significantly heavier ($p<0.01$) than the two CS groups (CS/CS and CS/GH).

Prenatal:Postnatal Litter-size: Similar multiple comparisons were made among the four groups to examine the ratio prenatal:postnatal litter-size. The analysis established that the ratios were not significantly different ($p>0.05$) between the two hormone-treated groups of mothers (GH/GH and GH/CS) and the two control groups of mothers (CS/CS and CS/GH), nor were there any differences between the two groups rearing offspring from the hormone-treated mothers (GH/GH and CS/GH) and those with offspring from control mothers (GH/CS and CS/CS).

Autopsy of Mothers: In addition to their increased body-weight on post-natal Day 40 (timed from conception) GH mothers were also significantly longer from nose to anus ($p<0.01$) and from nose to tail-tip ($p<0.025$); the increase in tail length was not significant ($p>0.05$). Significant increases in wet liver weight ($p<0.025$) and wet kidney weight ($p<0.0005$) were also found in the GH group (Table XXVIII). In the GH group, splanchnomegaly of the kidneys was evidenced by a significant increase ($p<0.05$) in kidney weight per unit body-weight (GH: 719.764 ± 17.580 mg./100 gm.; CS: 673.808 ± 11.699 mg./100 gm.), whereas the increase in liver weight appeared to be proportional to body weight and the ratio was not significantly different between groups (GH: 3793.744 ± 56.110 mg./100 gm.; CS: 3735.754 ± 82.400 mg./100 gm.).

Maternal Behaviour

Nest-Time:--Continuous records of nest-time, together with the frequency of nesting periods, were available for all of the sixteen mothers studied. Figure 38 shows daily nest-time (dated from parturition) for each of the four groups. In Figure 39, the data are collapsed into the two groups of eight mothers and group mean daily nest-times are shown dated from parturition. Because of the extended gestation period in the GH group, the data are also shown dated from conception so that in Figure 40 the GH curve is moved one day forwards. Both groups show a decline in nest-time through the period studied, and whether the measures are timed from parturition or from conception GH mothers show consistently less time in the nest compared with the CS mothers.

Total time spent on the nest over postnatal Days 2-15 (timed from parturition) for the GH group was 5745 ± 201 min. which was significantly less ($p < 0.01$) than the total time for the CS group, 6866 ± 237 min. Total time spent nursing over post-conception Days 24-36 was 5469 ± 149 min. for the GH mothers, and 5906 ± 208 min. for the CS mothers, the difference bordering on statistical significance ($0.1 > p > 0.05$).

Total nursing time over Days 2-15 postpartum was negatively correlated with mean maternal body-weight over this period ($r = -0.62$, $p < 0.01$). The same measures were also negatively correlated ($r = 0.35$) when maternal body-weight was timed from conception (Days 24-36) but not significantly so ($p > 0.05$).

Frequency of nest-periods:--The same procedure was followed

for the analysis of daily frequency of nest-periods. The data were expressed with reference to the day of parturition (Figure 41, lower) and to the day of conception (Figure 41, upper). For both analyses, no consistent differences are apparent between the two groups. For both groups of mothers, mean daily frequency showed a decline from the 40-50 range in the early postpartum period to values below 30 by the end of the second week.

Observations on the Development of the Offspring

On each of the following measures of maturation, the data are analysed in two ways: in the first analysis the respective postnatal ages of all pups are dated from conception so that all litters are considered born at the normal time on Day 21. In the second analysis, postnatal age is dated from the day of birth; in this case litters born on Day 21 are considered 0 days old as are litters born on Day 22.

Body-weight:--Mean pup weight[1] gain was relatively invariant between the four groups over the first 20 postnatal days (Figure 42). Despite the very close similarity in growth rate, it appears that after the first postnatal week, the two groups reared by the hormone-treated mothers (GH/GH and GH/CS) showed a slightly higher rate of growth than did the two groups reared by control mothers (CS/CS and CS/GH).

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[1]Mean pup weight for each litter was estimated from the mean of the mean weight of the males and the mean weight of the females at any age: $\frac{1}{2}\left[\bar{X}_{♀} + \bar{X}_{♂}\right]$

The rate of pup growth in all four groups appeared to be approximately linear during this period and a comparison of the slopes of the regression lines through Days 2-20 yielded, for the two hormone groups of mothers (GH/GH and GH/CS), respective values of 7.789 and 7.591, while for the control groups of mothers (CS/CS and CS/GH) the values were 7.294 and 7.245 respectively although the difference between the two sets of slopes was not significant ($p > 0.05$). Thus, it appeared that pup growth rate, when timed from conception, was slightly influenced by the postnatal foster mother and apparently not by the prenatal treatment.

When pup age was dated from birth, a slight "prenatal" influence was apparent. A multiple comparison of mean pup weights at postnatal Day 15 showed the two groups of GH pups ($\bar{X}_{GH/GH}$: 34.56 gm., $\bar{X}_{CS/GH}$: 32.78 gm.) to be slightly heavier than the two groups of CS pups ($\bar{X}_{CS/CS}$: 30.81 gm., $\bar{X}_{GH/CS}$: 31.90 gm.); the comparison bordered on statistical significance ($0.1 > p > 0.05$).

Mean pup weight at postnatal Day 15, when timed from conception, was positively correlated with mean maternal body-weight over the first two weeks postpartum but not significantly so ($r = +0.35$; $p > 0.1$), and the same was true when mean pup weight at 15 days was timed from birth ($r = +0.42$; $p > 0.1$).

The correlations between natural litter of the foster mother and mean pup weight at Day 15 (timed from conception or from parturition) were not significant ($p > 0.1$) for all sixteen mothers, or for the eight

GH or the eight CS mothers considered separately.

Eye-opening:--When pup age was dated from conception, no consistent differences between the groups were apparent that could be attributed to either prenatal or postnatal treatments. Group mean ages[1] at which first eye-opening occurred were: GH/GH: 14.66 ± 0.25; GH/CS: 15.16 ± 0.25; CS/CS: 14.91 ± 0.42; CS/GH: 15.09 ± 0.18.

When eye opening was dated from birth, however, multiple comparisons among the four groups yielded a significant difference ($p < 0.05$) of about one day between the groups containing GH pups (GH/GH and CS/GH, combined mean: 14.00 ± 0.12) and the groups containing CS pups (CS/CS and GH/CS, combined mean: 15.03 ± 0.14). When the data for all pups were pooled and dated from conception no noticeable effects due to sex were apparent. (Figure 43).

Startle reflex:--When pup age was dated from conception, no consistent differences due to either prenatal or postnatal treatment were apparent. Mean age for the litters reared by GH mothers (GH/GH and GH/CS) was 11.84 ± 0.08, and for those reared by CS mothers (CS/CS and CS/GH) 11.81 ± 0.05. Mean age for pups originally from GH mothers (GH/GH and CS/GH) was 11.83 ± 0.03, and for those from CS mothers (CS/CS and GH/CS) 11.83 ± 0.05.

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[1] As in the previous experiment, the mean of the litter of eight is considered as a statistical sample of one; the group mean on each of these measures of development is based on four means.

When pup age was timed from parturition, however, a multiple comparisons test on the log-transformed mean litter ages yielded a significant difference ($p < 0.01$) of about one day. Litters originally from GH mothers (GH/GH and CS/GH) had a mean age of 10.95 ± 0.14 against 11.83 ± 0.05 for the litters from CS mothers (CS/CS and GH/CS).

When the data from all pups was pooled as before, a small effect due to sex was apparent (Figure 43). With numbers in both groups equal (64), 17 females (27%) exhibited the reflex on the critical Day 11 compared with 4 males (6%).

Free-Fall Righting reflex:--A similar pattern was evident for the development of this reflex. When pup age was dated from conception, no effects ot prenatal or postnatal treatments were evident. Mean age for litters reared by GH mothers was 17.63 ± 0.21 compared with 17.95 ± 0.28 for litters reared by CS mothers. Mean age for pups originally from GH mothers was 17.84 ± 0.31 against 17.73 ± 0.17 for pups from CS mothers.

When pup age was dated from parturition, pups from GH mothers showed a significantly ($p < 0.05$) earlier appearance of the reflex: GH/GH and CS/GH combined mean: 16.97 ± 0.31; CS/CS and GH/CS combined mean: 17.73 ± 0.17.

A marginal difference due to sex was apparent when the data for all pups was pooled (Figure 43).

Vaginal Opening:--No consistent differences between the groups

were apparent in the age at which vaginal opening occurred. Dated from conception, mean age for the females reared by GH mothers (GH/GH and GH/CS) was 37.47 ± 1.22, compared with 38.53 ± 1.13 for those reared by CS mothers (CS/CS and CS/GH). Neither were there any significant differences when pup age was dated from parturition. Pups born to GH mothers showed, on average, over two days precocity in comparison to pups from CS mothers but the difference did not achieve significance (GH/GH and CS/GH combined mean: 36.344 ± 1.06; CS/CS and GH/CS combined mean: $38,78 \pm 1.32$; $p > 0.1$).

Female Brain Assay:--The results of the female brain assay at postnatal Day 56 (timed from parturition) are shown in Table XXXIa. Multiple comparisons among the four groups revealed no significant differences due to either prenatal or postnatal treatments.

The data resulting from an analysis based on postnatal treatment i.e., effect of foster mother, is summarised in the upper part of Table XXXIb, and the analysis based on prenatal treatment, i.e., prenatal origin of offspring, in the lower part of the table. Direct comparisons by t-test yielded no significant differences ($p > 0.1$) in any of these measures that could be ascribed to either prenatal or postnatal treatments.

When compared with conception-dated mean pup-weight at Day 15 all of the measures correlated positively, but in no case significantly; (body-weight: $r = +0.36$, $p > 0.05$; brain-weight: $r = +0.21$, $p > 0.1$;

brain DNA: $r = +0.29$, $p > 0.1$; brain protein: $r = +0.32$, $p > 0.1$). The results were similar when pup-weight was dated from parturition: (body-weight: $r = +0.47$, $p > 0.05$; brain-weight: $r = +0.41$, $p > 0.1$; brain DNA: $r = +0.29$, $p > 0.1$; brain protein: $r = +0.24$, $p > 0.1$).

Open-field Behaviour of Males:--Open-field scores for the four groups of males over Days 75-80 are summarised in Table XXXII. Multiple comparisons among the four groups revealed no significant effects that could be attributed to either prenatal or postnatal treatments.

Litter mean activity scores were negatively correlated with total nursing time over Days 2-15 ($r = -0.26$) and with total nursing time over post-conception days 24-36 ($r = -0.23$) but in neither case was statistical significance achieved ($p > 0.1$).

The use of the defecation scores in this situation is probably invalidated by the fact that only those boluses deposited in the open field were counted. Thus, a subject with a long latency for entrance into the field may have defecated in the waiting-box and subsequently defecated less in the open-field. This conclusion is supported by the absence of any correlation between defecation in the field and activity ($r = -0.007$).

That the latency measure itself can be considered as a substitute index of "emotionality" is suggested by a significant inverse correlation with activity ($r = -0.53$, $p < 0.05$). No significant correlations were found, however, between the latency measure and nursing time over Days 2-15 ($r = +0.21$, $p > 0.1$) or with nursing time over post-conception days 24-36 ($r = +0.25$, $p > 0.1$).

DISCUSSION

The results of Experiment VIIIa, although confounded by extraneous factors, provide support for the finding previously reported, of a growth hormone-induced increase in maternal body-weight over and above that which normally occurs during pregnancy (Teel, 1926; Hain, 1932a; Watts, 1935; Frazer, Huggett, and Wohlzogen, 1949; Nixon, 1954; Experiments II, III, IV, of present study). The nature of this weight increase is discussed below under Experiment VIIIb.

Of particular interest was the extension of normal gestation that occurred under the influence of the two hormone preparations. Apparently, this effect was not due to other conditions in the experiment as the control mothers that had successful pregnancies delivered their litters after a normal gestation period. Further, it appeared that the two hormone preparations, although administered at equivalent dose-levels,[1] produced different effects on the gestation period. While those mothers receiving the bovine preparation showed an extension of gestation of about one day and appeared (with one exception) to deliver their litters normally, those receiving the ovine preparation underwent more prolonged gestation

[1] Although the potency of the two preparations was adjusted to equality on the basis of the assay information provided by NIH, true equilibration is not guaranteed as the NIH estimated is based on the mean potency of several assays.

which was associated with fetal mortality. The several possibilities for this effect are: qualitative differences between ovine and bovine preparations; actual differences in potency; differential contamination by other pituitary hormones.

Before discussing the results of Experiment VIIIb, a preliminary comment would be appropriate concerning the comparability of Experiments VII and VIIIb. The fundamental objectives in both experiments were identical and, indeed, it was anticipated that the design of both experiments would also be the same. However, the fact that growth hormone-treatment extended gestation forced a modification of the cross-fostering procedure and therefore of the experimental design. This, together with other procedural changes (see above) precludes any direct comparison between the studies even though some effects were replicated.

In Experiment VIIIb, the effect of growth hormone on maternal body-weight during pregnancy was confirmed. Observations continued into the postpartum period established that a significant differential in body-weight was maintained, but by postnatal Day 40 the mean difference was less than half of that which obtained on prenatal Day 21. Although the increase in body-weight during pregnancy has been observed by others (see above) using crude preparations of the hormone, the effect has been described in only two of the studies that have employed purified preparations of the hormone (Nixon, 1954; Tuchmann-Duplessis and Mercier-Parot, 1955). In the absence of any direct

measures of growth, Hain (1932a) surmised that the weight-increase was due to the deposition of fat. She also suggested that increased mammary growth was not responsible for the effect as, during the period covered by her injections (first six days of pregnancy), mammary development was negligible.

Later work in the non-pregnant rat by Greenbaum (1953), and Greenbaum and McLean (1953a, b), suggests that, under the influence of growth hormone, fat mobilisation occurs and that its oxidative metabolism provides a source of energy which spares the dietary proteins and makes them available for the formation of new tissue. In the present study, measurements on the mothers at postnatal Day 40 provide a clear demonstration that a genuine growth occurs in response to the hormone during pregnancy. It is possible that the additional fat depots that are laid down in early pregnancy (Beaton, Beare, Heh Ryu, and McHenry, 1954) may augment the responsiveness to the hormone. The finding, in the present study, of a proportional increase in liver weight and a splanchnomegalic increase in kidney weight contrasts with the observations of Bates, Milkovic, and Garrison (1964) in the non-pregnant rat. Following 10 daily injections of 10 mg. of growth hormone, these workers found a splanchnomegalic increase in liver-weight but a proportional increase in kidney-weight. If the difference in organ growth observed in these two studies is not due to effects of the hormone regime and dose-level, it may be due to a synergistic relationship between growth hormone and

other hormones during pregnancy.

Although the increase in maternal weight that Hain (1932a) observed may not have involved an increase in mammary tissue, it is possible that the hormone regime employed in the present study stimulated mammary growth. Development of the mammary glands is extremely rapid during the second two weeks of pregnancy (see Schmidt, 1971), and Moon (1965) has reported a significant increase in mammary gland DNA by prenatal Day 20 following daily injections of 2 mg. of growth hormone over Days 3-19. Thus, part of the maternal body-weight increase may have been due to a specific increase in mammary tissue. This possibility receives some support from the postnatal pattern of body-weight loss in the GH group in the present study. Whereas in Experiment VII, the mean difference in body-weight between heavy and light mothers dropped from 33 gm. on prenatal Day 21 to 18 gm. by postnatal Day 40, the difference between GH and CS mothers dropped from 50 gm. on Day 21 to 22 gm. by Day 40. As total involution of the mammary glands was evident at autopsy on postnatal Day 40, it appears that a greater proportion of the weight change in the present study may have involved the mammary glands.

An alternative possibility is that exogenous GH does not have a selective mammogenic effect but, instead, achieves the increase in mammary gland DNA that Moon (1965) observed, by inducing a higher rate of growth in the mother. This may have been associated with a

similar allometric increase in mammary gland growth to that which has been observed in the normally-growing rat (Sinha and Tucker, 1966; see discussion, Experiment VII). To summarise, the evidence suggests that either through a selective effect of GH on the developing mammary gland, or by an allometric increase in mammary gland growth accompanying the GH-induced higher rate of growth, increased mammary growth would be expected and may have been partly responsible for the increase in maternal body-weight during pregnancy.

The indication in Experiment VIIIa and the unequivocal demonstration in Experiment VIIIb of an increase in gestation time in mothers receiving GH is of considerable interest. In some of the earlier studies in which crude preparations of the hormone were used (Teel, 1926; Hain, 1932b; Sontag and Munson, 1934; Watts, 1935; Zamenhof, 1942) extended gestation was reported and was attributed to either a delay in implantation or pituitary gonadotrophic contaminants. Surprisingly, in only two of the many studies that have been conducted since the purified crystalline preparations became available in the 1940s has such an effect been reported (Nixon, 1954; Tuchmann-Duplessis and Mercier-Parot, 1955). An explanation for this may be found, perhaps, in the normal definition of "term." Although the gestation period of the rat is reported to be 21 days (Altman and Dittmer, 1962) a margin of one day either side of this estimate would be considered within normal limits, and Dieterlen (1963) gives the range of variation as 20-24 days. As the

exact time of parturition is not usually recorded in most studies, it would not be surprising if a difference of about one day escaped notice.

Before considering some of the possible mechanisms by which growth hormone treatment may extend gestation, some preliminary comments are in order. First, prolongation of gestation may have been caused by contamination with other pituitary hormones. Earlier preparations of the hormone were contaminated with adrenocorticotrophin and thyrotrophin (see Clendinnen and Eayrs, 1961), and although present preparations of growth hormone supplied by NIH are of a high degree of purity, it is possible that there could be a cumulative effect of trace contaminants. Second, the degree of extension of gestation in GH-treated mothers may not reflect the absolute influence of the factor responsible for the delay. It has been demonstrated here and elsewhere that parturition bears a strong relationship to the photoperiod. Thus, were the delaying influence sufficient to hold off parturition only for a few hours until the beginning of the next dark cycle, the influence of photoperiod might be strong enough to generate a further delay so that parturition is held over into the beginning of the next photoperiod. This may have been the case in the present study.

The mechanism by which prenatal treatment with growth hormone extends gestation is presently obscure but it seems clear, at least, that the phenomenon is not related to maternal body-weight at the time of parturition. In Experiment VII, using animals of the same strain

and bred under the same conditions, no relationship was found between maternal body-weight and gestation period. The range of maternal body-weight was substantially wider than the differential established in the present study through the effect of growth hormone. It is also clear that the effect is not due to a reduction in litter-size in the GH group (see below).

It has been demonstrated by several groups (see Pepe and Rothchild, 1972) that parturition in the rat is preceded by a decline in progesterone secretion, resulting from the regression of the corpora lutea. Although the pituitary gonadotrophin, luteinising hormone (LH) has been considered as the specific luteotrophic agent (Raj and Mougdal, 1970), the demonstration that hypophysectomy on Day 12 of pregnancy results in a slower than normal decline in serum progesterone near term and extended gestation (see also Tuchmann-Duplessis and Mercier-Parot, 1955) suggests a non-essential role for pituitary LH (Pepe and Rothchild, 1972). Alternatively, it seems that the sustaining luteotrophic stimulus may be placental in origin (Linkie and Ninswender, 1971). The immunological similarity between growth hormone and this chorionic luteotrophin (see Pecile and Muller, 1966; earlier discussion in Section I) raises the possibility that exogenous GH may mimic the luteotrophic action of the placental hormone. The administration of GH in late gestation (up to and including Day 20 in the present study) may therefore act to extend the progesterone blockade of parturition.

A second possibility is that the delay in parturition may not have been due to a chronic or acute excess of GH but instead to an acute reduction in endogenous GH at normal term resulting from a "short" feedback effect of the exogenous GH (Krulich and McCann, 1966; see also Motta, Fraschini and Martini, 1969). In this regard, the study by Pepe and Rothchild (1972) is of interest, in that maternal hypophysectomy resulted in prolongation of pregnancy, and was associated with a slower than normal decline in serum progesterone level after Day 19. Against an "acute" explanation however is the finding that gestation was prolonged when growth hormone-treatment was discontinued on Day 15 of pregnancy (Tuchmann-Duplessis and Mercier-Parot, 1955).

A third possibility involves both the fetal and maternal adrenal glands. A facilitative effect of GH on adrenal gland weight in the adult animal has been reported in several studies (see Mangili, Motta, and Martini, 1966), and depressed fetal adrenal gland growth is reported to occur when the output of maternal corticosteroids is high (see Jost and Picon, 1970). The involvement of fetal corticosteroids in prostaglandin ($PGF_2\alpha$) synthesis (see Liggins, 1972) suggests that a reduction of fetal corticosteroid output might reduce prostaglandin synthesis. Luteolysis might therefore be delayed with a consequent extension of the progesterone blockade of parturition. Although there are reports of adrenal gland hypertrophy in the newborn offspring of mothers receiving GH preparations during pregnancy (Hultquist and Engfeldt, 1949; Engfeldt and

Hultquist, 1953) it seems likely from other studies that this effect was due to postmaturity (see Boe, 1938; Eguchi and Ariyuki, 1963; Thliveris and Connell, 1973). In fact, the adrenal hypertrophy observed in postmaturity by Eguchi and Ariyuki (1963) was associated with a reduced content of ascorbic acid, which indicates reduced adrenal gland activity (Sayers, Sayers, Liang and Long, 1946). Thus, the effect of daily injections of GH may have been to increase maternal adrenal gland weight and maternal corticosteroid output. This could have caused a suppression of fetal adrenal gland growth, and a reduction in fetal corticosteroid output, resulting in reduced prostaglandin synthesis and a consequent delay in parturition.

It is also possible that $PGF_2\alpha$ is involved more directly. Li (1956) has reported a direct action of GH on the uterus, and Pharriss (1970) has suggested that local changes in the uterus may influence the blood supply to the ovaries, and modify a local vasoconstrictor effect of $PGF_2\alpha$ on ovarian blood supply. Thus, exogenous GH may effect local uterine changes which delay the subsequent luteolysis and regression of the corpus luteum theorised to result from a prostaglandin-induced reduction in arterial perfusion of the ovaries.

One final possibility is that growth hormone has a prolactin-like luteotrophic action. Growth hormone and prolactin have similar biologic actions and it is possible that, like prolactin, growth hormone may act to sustain the release of progesterone from the corpora lutea, or from

the adrenal gland (see Piva, Gagliano, Motta, and Martini, 1973), to support the blockade of parturition.

The pattern of births in the CS group essentially supports the findings of Experiment VII; timing of parturition was again related to the photoperiod, and negative regressions were found between litter-size and birth-weight, and between litter-size and length of gestation (although the effect was weaker in the latter). In addition, the observation first made by King (1915), and later by others (Angervall, 1959; Benson and Morris, 1971; Smart, Adlard, and Dobbing, 1972) of an influence of sex on birth-weight, receives further confirmation here.

In the GH group, the prolonged gestation appears to have had a slightly disruptive effect on these relationships. Although the influence of sex on birth-weight, and the relationship of birth-weight to litter-size, were both confirmed, not all births took place in the light phase, and no relationship was found between length of gestation and litter-size.

With regard to litter-size, previous reports have suggested that the prenatal administration of GH may result in a diminuition in the size of the litter (Barns and Swyer, 1952; Campbell, Innes and Kosterlitz, 1953; Cotes, 1954; Nixon, 1954), and the possibility is raised that both fetal "gigantism," and the extension of gestation, may be secondary to this effect. In the present study, however, as in those described earlier (Experiments II, III, IV), no effect of GH on litter-size has been found.

The analysis of maternal behaviour yielded results that were in general accord with those of Experiment VII. For all mothers, daily nest-time showed a decline, and frequency of nest-periods a slight decline, through the period studied. As before, the heavier group of mothers (GH) spent less time with their litters, and maternal body-weight was negatively correlated with nest-time. That the correlation was statistically significant when nest-time was dated from parturition, and not when it was dated from conception, raises the question of from when nest-time should be dated. The synchronous relationship that has been demonstrated between nest-time and age of litter (Grota, 1973), together with the possibility that nest-time is related to the stage of homoiothermic development of the litter (Experiment VII), suggests that nest-time should be dated with respect to the conceptual age of the litter. However, while there may prevail an overall synchrony between nest-time and age of litter, the relationship may not be so clear in the early postpartum period. Although parturition is presently viewed as the pivotal event in the initiation of milk secretion (see Schmidt, 1971), it is not clear what effect extended gestation has upon the process. Also, the effect upon the fetus of spending almost a full day in utero past the time when it was sufficiently mature to be born, is not known. It is possible that homoiothermic development of post-mature offspring is different from that of normally-born offspring. Thus, if temperature-regulation is involved in the regulation of nest-time,

changes in the homoiothermic development of the litter, if only in the early stages of the postpartum period, would transiently alter the synchrony referred to above.

A second major problem concerns the causal basis of the lowered nest-time in the GH group. If, as it appears, exogenous GH is mammogenic during pregnancy, then an essential parallel exists between the heavy mothers of Experiment VII, and the GH mothers of the present study. Theoretically, both groups would have had a relatively larger amount of mammary tissue in the postpartum period (compared with their respective comparison groups), perhaps associated with increases in milk-output. This, in turn, may have determined the amount of time spent with the litter (see earlier discussion, Experiment VI). On the basis of the temperature-regulation theory of nursing that has been proposed (Experiment VII), the increased body-weight of the GH mother in the postpartum period would be expected to result in a lowered nest-time.

Where the early development of the offspring is concerned, it is clear from the present data that pups born to GH mothers show no precocity when compared with those from CS mothers when pup-age is dated from conception. When postnatal age is dated from parturition, however, significant gains are observed, both in physical and CNS maturation. This raises the question of dating of developmental events.

The two obvious possibilities, as Barrow (1970) points out, are:

(i) developmental events occur at a genetically-predetermined point in time, and the event of parturition is without effect on this timing, and (ii) postnatal events may be initiated by, and therefore bear a temporal relationship with, parturition. A third possibility might involve some interaction between the two such that a given developmental event may be genetically programmed to occur in a given time interval but will be influenced by the timing of parturition. Further, there may not necessarily exist a simple temporal relationship between parturition and those events that are initiated by it.

In attempting to divide developmental events into those which are timed from conception and those timed from parturition, it might be expected that developmental changes associated with those systems that undergo a substantial change in the transformation from in utero to ex utero life, would bear some temporal contingency upon parturition. Thus, Barrow (1970) has demonstrated that closure of the ductus ateriosus, which normally occurs within 45 min. of parturition, does not occur in utero when parturition is delayed by up to two days. Preliminary observations by Henderson (1973) on hooded rats indicate that the initial population increase in fungiform papillae of the neonatal tongue is delayed in the offspring of mothers normally delivering on Day 22, compared with those of mothers delivering on Day 21. Also decussation of the corticospinal tracts occurs within 24 hr. of parturition (DeMeyer, 1967) and this event may similarly be stimulus-bound,

i.e., to the dramatic change in afferent input following parturition.

For developmental events that are associated with systems that do not undergo dramatic change at birth, it seems more likely that they are timed from conception. Barrow (1970) studied the timing of hood-pigmentation in the Long-Evens rat, and found that it occurred on post-conception Day 24, whether parturition was normal or extended by progesterone.

With developmental events that are programmed to occur more distantly from parturition, their time of appearance is presumably subject to environmental influences. For example, in Experiment VII it was demonstrated that the timing of reflexes, normally appearing in the second to third week postpartum was influenced by the foster mother.

With insufficient experimental data to justify any firm conclusions on this important aspect of development, it would be advisable to adopt the most parsimonious explanation. In the present study, a difference of approximately one day was found between pups from GH mothers and those from CS mothers when developmental criteria were timed from parturition, and the gestation periods of these two groups of mothers differed by about the same margin. Therefore, it seems reasonable to conclude that these events should normally be timed from conception. In support of this conclusion, Gainer (1974) has established essentially similar findings to those of the present study. The offspring from untreated mothers who delivered on Days 21 or 22, when compared on

these developmental criteria, showed differences that could be accounted for entirely by the difference in gestation period.

One difficulty in the present study is that, with postnatal development timed from conception, no obvious influence of the foster mother was apparent on pup development (with the exception of body-weight). Neither was it possible to demonstrate an effect of prenatal:postnatal litter-size ratio of foster mother on pup growth. If the criterion is adopted that postnatal development is best timed from conception, then it should be possible, on the basis of the results obtained in Experiment VII, to demonstrate an influence of foster mother. The only parameter for which such an influence was apparent was pup body-weight. The two groups reared by GH mothers showed a slightly higher rate of gain through the first two postpartum weeks than did the two groups reared by CS mothers, and pup weight by Day 15 was positively correlated with maternal body-weight. Although this is the same pattern as that observed in Experiment VII, the effect of foster mother on pup growth is not as strong as would be predicted on the basis of the difference in maternal body-weight between GH and CS groups. In Experiment VII, the body-weight difference between heavy and light mothers was 30 gm. or less through the postpartum period whereas in the present study, the difference was greater.

Perhaps the obvious source of this inconsistency lies in the influence of GH on gestation period and in the fact that the experimental design

had to be changed to accommodate this effect. In setting up the four postnatal conditions it was necessary in some cases to place pups of one-day postnatal age with mothers who had just given birth and, in other cases, to give pups who had just been born to mothers who had given birth one day previously. Thus, it was not possible to replicate the conditions of Experiment VII, and it was necessary in two of the four groups to alter the normal temporal synchrony between mother and litter in the postpartum period.

A second source of variation may have arisen from the different degree of "handling" in the two studies. In Experiment VII, pups were weighed at five-day intervals from birth, whereas, in the present study, weighing was performed daily. If, as would be expected, a ceiling effect operates in the "early handling" phenomenon (see earlier discussion) then the increased amount of handling in the present study may have eliminated any differences due to foster mother by differentially favouring the pups reared by CS mothers.

A third possibility is that the maternal body-weight differences established through GH treatment may not be qualitatively comparable to the ad libitum differences in maternal body-weight that prevailed in Experiment VII. Although there are, in both cases, superficial similarities in body-composition, and perhaps in the mammary glands, GH mothers may have been qualitatively different in other respects from the heavy mothers of Experiment VII.

Aside from the procedural changes that were made in the open-field testing, these same considerations may apply. Unfortunately, neither the results of the adult behaviour test, nor the observations on early development, are comparable in any absolute sense with those of Experiment VII. Although the two studies were completed within six months of each other, seasonal and other variations also preclude any direct comparisons.

SUMMARY

The findings and conclusions of the present study may be summarised as follows:

1. For the control group of mothers, parturition was related to photoperiod. Litter-size showed a negative regression on gestation time, and on birth weight, for litters born on Day 21. Birth-weight of male pups was greater than that of females.

2. Three different preparations of growth hormone, when administered from Days 7-20 of gestation, produced greater increases in maternal body-weight than normally occur during pregnancy, and all prolonged gestation. Ovine growth hormone produced a greater extension of gestation than did bovine growth hormone.

3. For mothers receiving a bovine preparation of growth hormone, a negative regression of birth-weight on litter-size was found but no relationship was apparent between litter-size and gestation time. Not all births occurred in the light phase, some occurred in the late part of the dark phase. Birth-weight of male pups was greater than that of females. No effect on litter-size was found.

4. Autopsy of mothers at postnatal Day 40 (post-conception Day 61) established that mothers receiving growth hormone during pregnancy had shown a genuine growth response. Liver weight increased proportionally to body-weight whereas kidney weight increases were disproportionate.

5. All mothers showed a decline in nest-time and in the frequency of nest-periods in the postpartum period. Growth hormone-treated mothers spent less time with their litters than did control mothers. Total time spent nursing was negatively correlated with maternal body-weight.

6. Offspring from growth hormone mothers exhibited precocious development compared with offspring from control mothers when pup age was dated from parturition. However, both groups of offspring showed comparable development when pup age was dated from conception. A slight precocity of females over males was

apparent across all groups for the startle reflex.

7. A marginal influence of foster mother was apparent on pup weight gain in the first two postnatal weeks. No influence of foster mother was apparent on any other developmental parameter.

8. No effects, due to either prenatal or postnatal treatment, were apparent with respect to body and brain development of female offspring at 56 days postpartum.

9. No effects, due to either prenatal or postnatal treatments, were apparent in male behaviour in the open field. Across all groups, a negative correlation was found between the latency to enter the field and subsequent activity in the field.

It was concluded that, while prenatal treatment with growth hormone resulted in an altered profile of maternal behaviour in the postpartum period, through alterations in the temporal synchrony between mother and litter and possibly other factors, the influence of foster mother on postnatal development and adult behaviour of the offspring was obscured.

Previous reports of precocious development of GH offspring are probably due to a small but significant increase in the gestation period of GH-treated mothers resulting in errors in dating of age from conception upon which the timing of developmental parameters appears to depend.

Table XXIX

Experimental design. For each of the four postnatal groups the first letters denote prenatal treatment of mother and the second letters the prenatal origin of the pups. GH = growth hormone; CS = control.

Table XXIX

Experimental Design

Postnatal Group	Prenatal Treatment		Number of Litters
	Mother	Pup	
GH/GH	GH	GH	4
GH/CS	GH	CS	4
CS/CS	CS	CS	4
CS/GH	CS	GH	4

Table XXX

Measurements at autopsy on eight growth hormone-treated (GH) mothers and eight control (CS) mothers on postnatal Day 40 (post-conception Day 61).

Table XXX

MEASUREMENTS AT AUTOPSY ON GROWTH HORMONE-TREATED (GH) AND SALINE INJECTED (CS) MOTHERS ON POSTNATAL DAY 40

	GH		CS			
Measurement	Mean	SE	Mean	SE	t	probability
Body-weight (gm.)	289.4	± 3.67	267.3	± 4.95	3.58	$p < .005$
NA* Length (cm.)	21.93	± 0.10	21.33	± 0.19	2.78	$p < .01$
Tail Length (cm.)	18.93	± 0.22	18.33	± 0.33	1.477	ns
Overall Length (cm.)	40.83	± 0.25	39.66	± 0.46	2.257	$p < .025$
Liver weight (gm.)	20.98	± 0.25	10.00	± 0.36	2.202	$p < .025$
Kidney weight (gm.)	2.08	± 0.04	1.80	± 0.04	4.432	$p < .0005$

*nose to anus

Table XXXI

Measurements at autopsy and brain assay of female offspring on postnatal Day 56. Upper table (a) shows mean values (±standard error) for the four groups. In the lower table (b) postnatal mother effects are compared in the first two rows, and prenatal treatment effects in the second two rows.

Table XXXI(a)

	Group Mean ± Standard Error			
	GH/GH	GH/CS	CS/CS	CS/GH
Body weight (gm.)	197.8 ± 4.1	179.8 ± 7.0	188.7 ± 6.9	178.2 ± 4.3
Brain weight (mg.)	1.816 ± 0.012	1.756 ± 0.012	1.786± 0.009	1.764± 0.015
Brain DNA (µg.)	1626.8 ± 30.7	1634.8 ± 24.5	1592.0 ± 31.9	1640.8 ± 17.0
Brain Protein(µg)	1688. ± 25	1682 ± 55	1638 ± 72	1611 ± 57

Table XXXI(b)

Groups		Body Weight	Brain Weight	Brain DNA	Brain Protein
GH/GH and GH/CS	$\bar{X}$	188.8	1.786	1630.8	1685
	SE	5.1	0.014	18.2	28
CS/CS and CS/GH	$\bar{X}$	183.4	1.775	1616.4	1625
	SE	6.6	0.017	19.1	43
GH/GH and CS/GH	$\bar{X}$	188.0	1.789	1633.8	1649
	SE	4.6	0.013	16.4	32.
CS/CS and GH/CS	$\bar{X}$	184.3	1.771	1613.4	1659
	SE	4.8	0.009	20.3	43

Table XXXII

Summary of open-field behaviour of male offspring over postnatal Days 75-80.

Table XXXII

Open-Field Summary

Measure	Group Mean (± Standard Error)			
	GH/GH	GH/CS	CS/CS	CS/GH
Latency to enter	72.4 ± 14.3	81.4 ± 6.9	69.4 ± 20.0	83.9 ± 14.0
Squares entered	75.1 ± 6.5	68.9 ± 3.4	73.9 ± 8.3	59.4 ± 5.9
Boli (in field)	0.4 ± 0.1	1.8 ± 0.6	1.8 ± 0.7	1.8 ± 0.7
Close-rear	18.5 ± 2.0	17.3 ± 1.6	19.1 ± 1.8	15.6 ± 0.8
Open-rear	8.2 ± 2.2	3.9 ± 0.4	7.9 ± 2.7	7.3 ± 2.0

Figure 36

Comparison of birth-times between control mothers of Experiment VII (26) and VIIIb (21) and growth hormone-treated mothers of Experiment VIIIb (9). 12-hour light (0800-2000 hr.) and dark (2000-0800 hr.) periods indicated on abscissa.

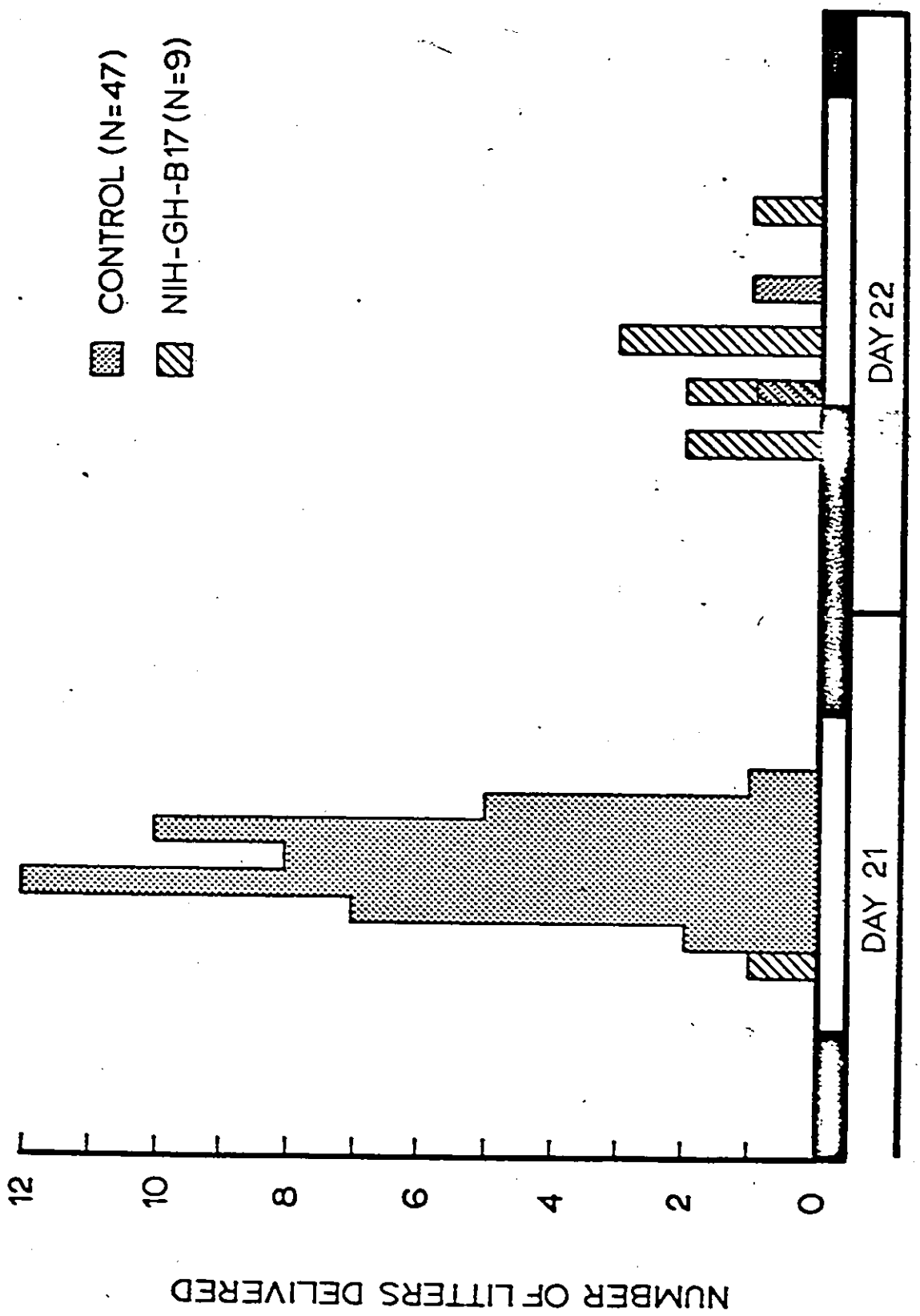
CONTROL (N=47)
NIH-GH-B17 (N=9)
DAY 22
DAY 21
12
10
8
6
4
2
0
NUMBER OF LITTERS DELIVERED

Figure 36

Figure 37

Maternal body-weight change through pregnancy and lactation for eight control mothers (open circles) and eight mothers treated with growth hormone (filled circles).

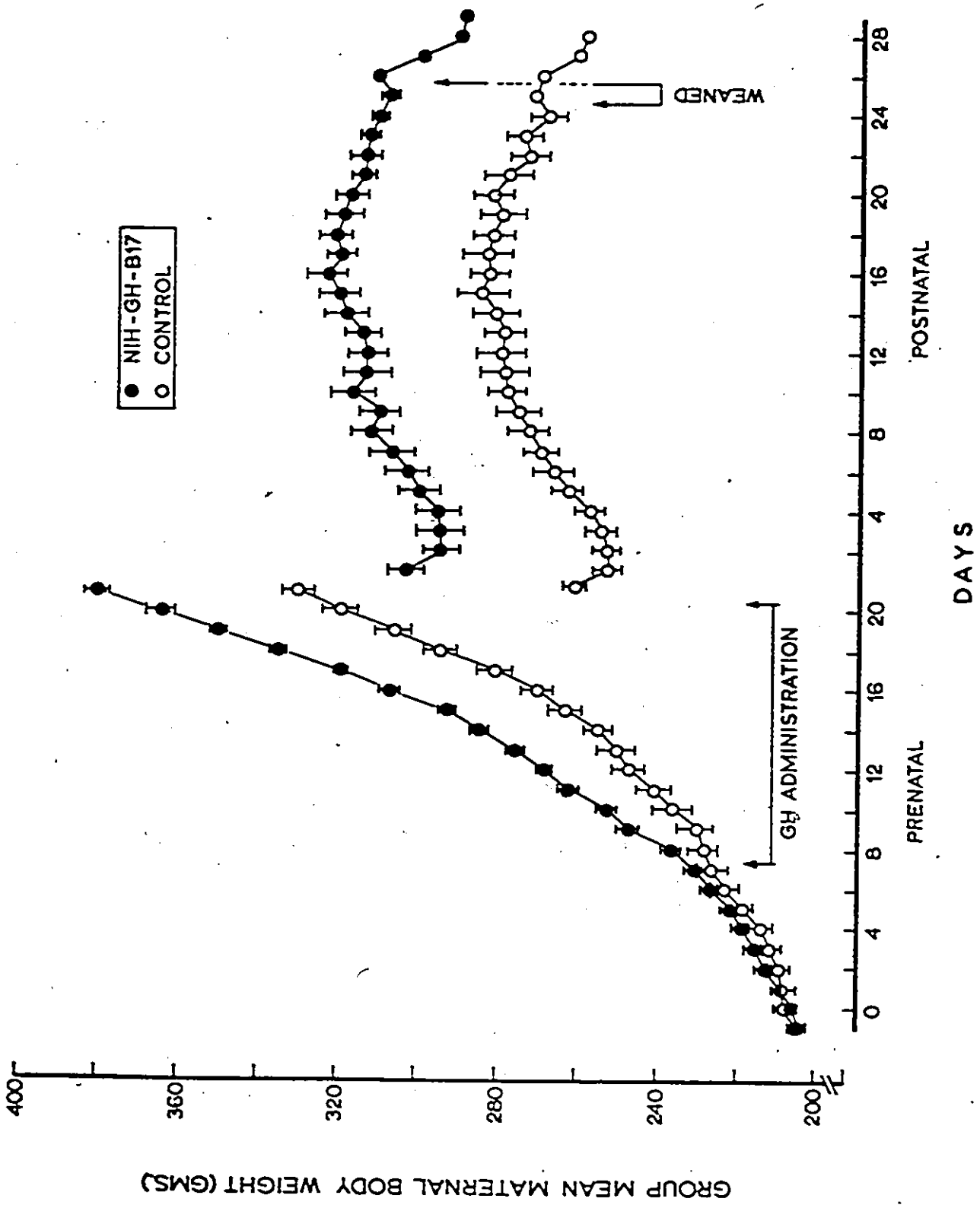
NIH-GH-B17
CONTROL
WEANED
GH ADMINISTRATION
PRENATAL
POSTNATAL
DAYS
GROUP MEAN MATERNAL BODY WEIGHT (GMS)
400
360
320
280
240
200
0
4
8
12
16
20
4
8
12
16
20
24
28

Figure 37

Figure 38

Group mean daily nest-time for each of the four groups.

Stage of lactation is timed from parturition (= Day 0).

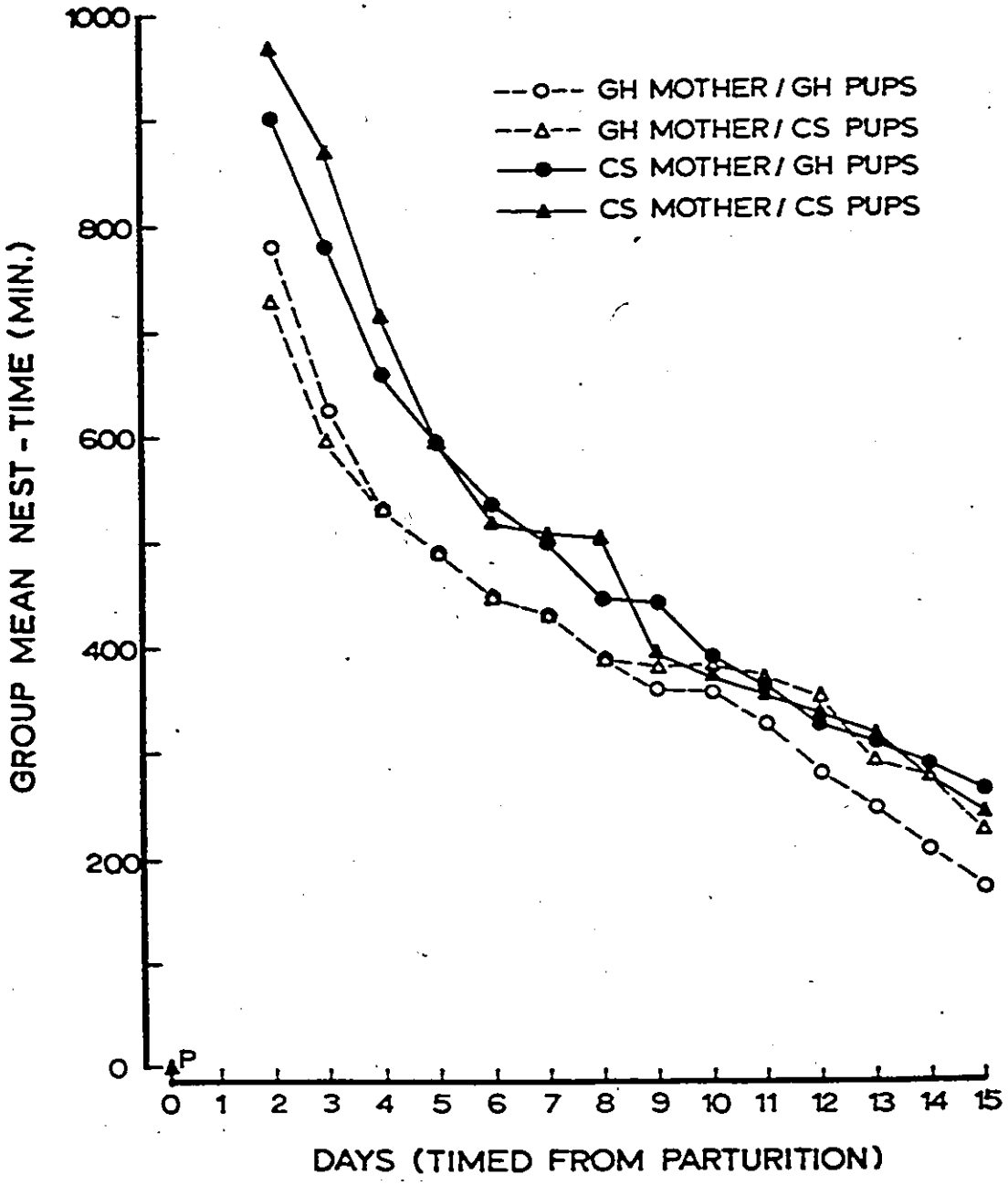
GH MOTHER / GH PUPS
GH MOTHER / CS PUPS
CS MOTHER / GH PUPS
CS MOTHER / CS PUPS
GROUP MEAN NEST-TIME (MIN.)
1000
800
600
400
200
0
P
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS (TIMED FROM PARTURITION)

Figure 38

Figure 39

Group mean daily nest-time for growth hormone-treated mothers (broken line) and control mothers (solid line). Stage of lactation is timed from day of parturition. Vertical bars indicate standard error of the mean.

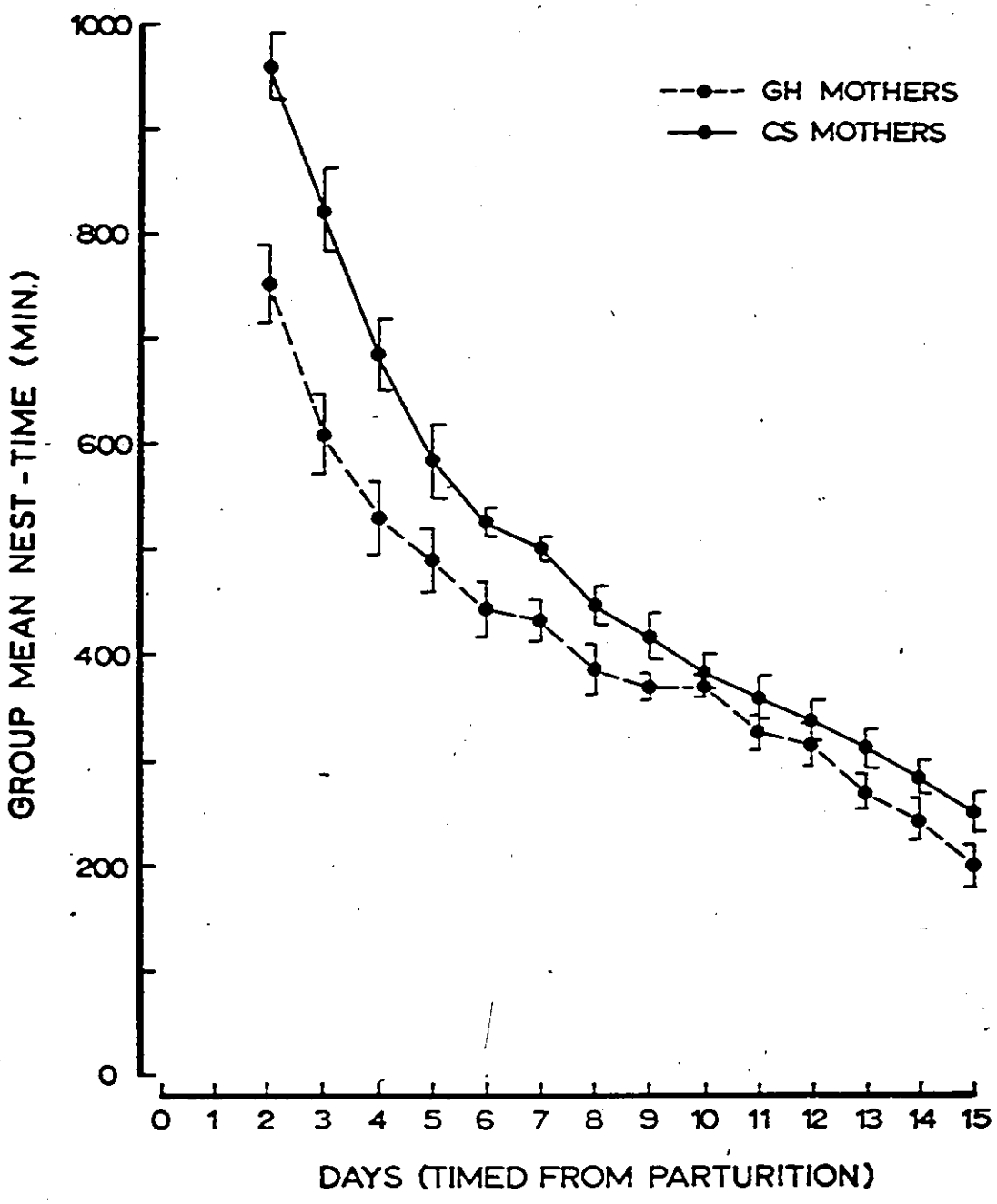
GH MOTHERS
CS MOTHERS
GROUP MEAN NEST-TIME (MIN.)
1000
800
600
400
200
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS (TIMED FROM PARTURITION)

Figure 39

Figure 40

Group mean daily nest-time for growth hormone-treated mothers (broken line) and control mothers (solid line). Stage of lactation is timed from onset of pregnancy. Vertical bars indicate standard error of mean.

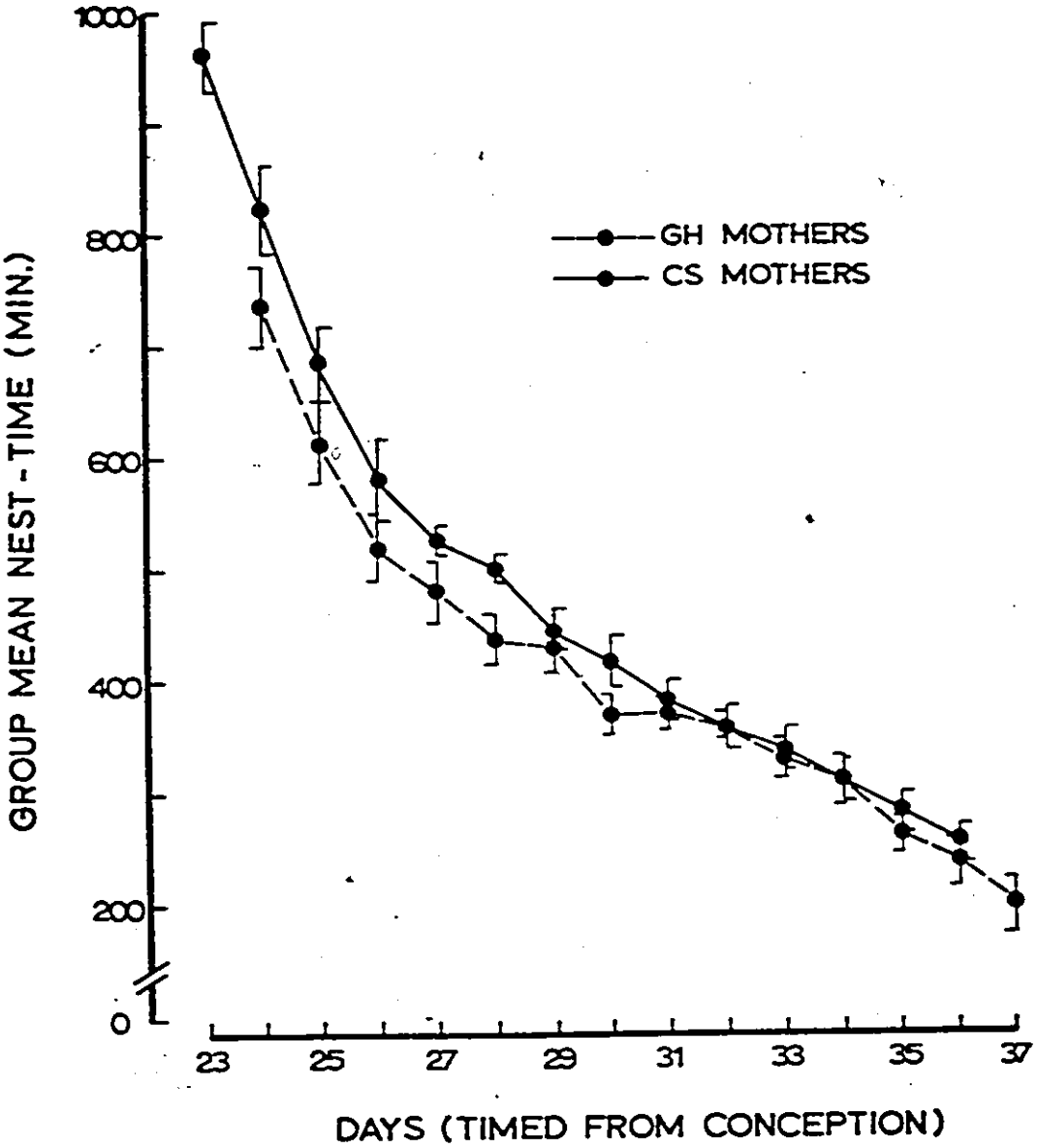
GROUP MEAN NEST-TIME (MIN.)
1000
800
600
400
200
0
GH MOTHERS
CS MOTHERS
23
25
27
29
31
33
35
37
DAYS (TIMED FROM CONCEPTION)

Figure 40

Figure 41

Group mean frequency of nest-periods for growth hormone-treated mothers (broken line) and control mothers (solid line) timed from conception (upper graph) or parturition (lower graph). Vertical bars indicate standard error of the mean.

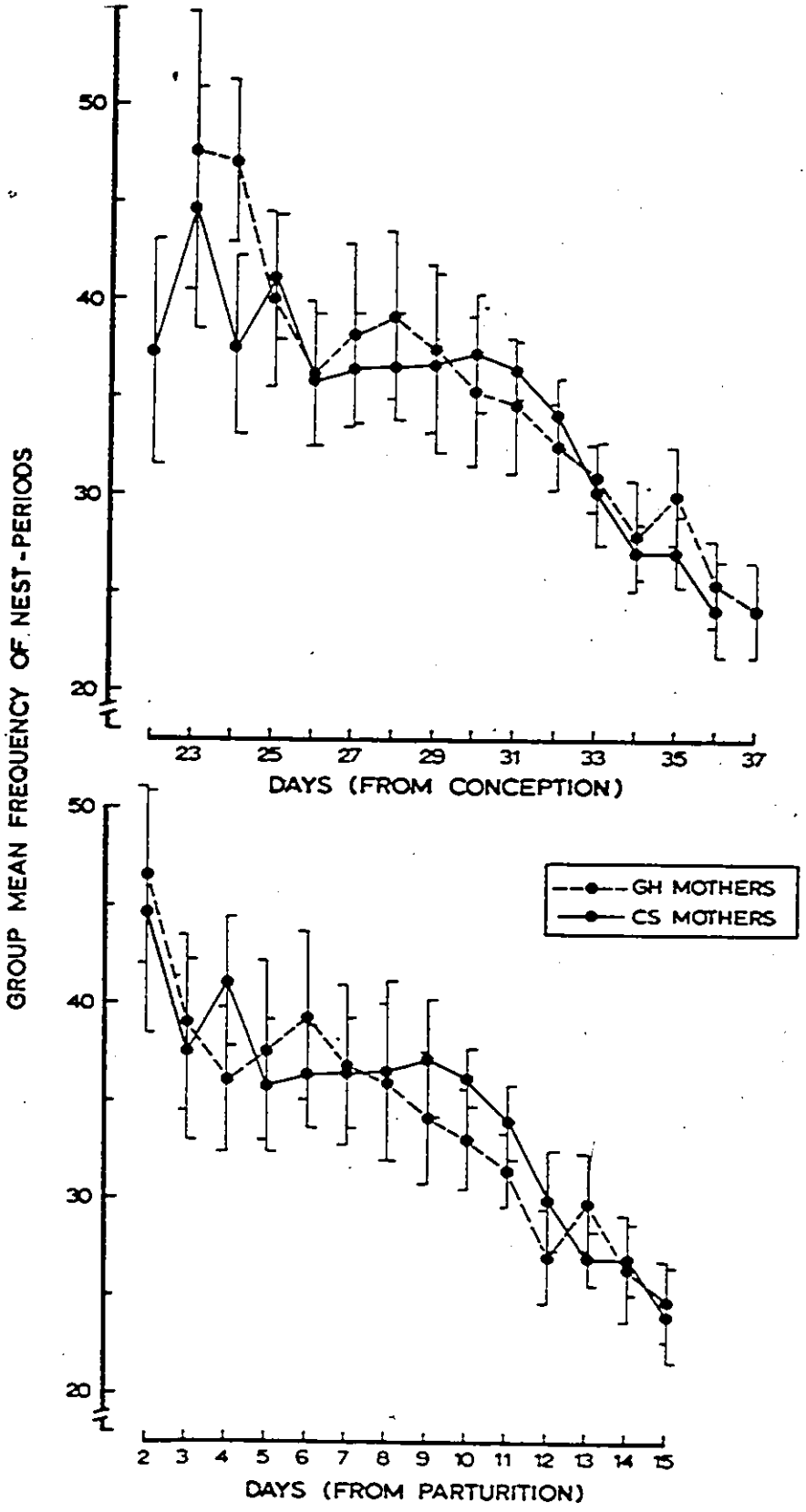
GROUP MEAN FREQUENCY OF NEST-PERIODS
50
40
30
20
23
25
27
29
31
33
35
37
DAYS (FROM CONCEPTION)
GH MOTHERS
CS MOTHERS
2
3
4
5
6
7
8
9
10
11
12
13
14
15
DAYS (FROM PARTURITION)

Figure 41

Figure 42

Group mean pup-weight for each of the four groups in the postnatal period. Growth hormone mothers: broken lines; control mothers: solid lines.

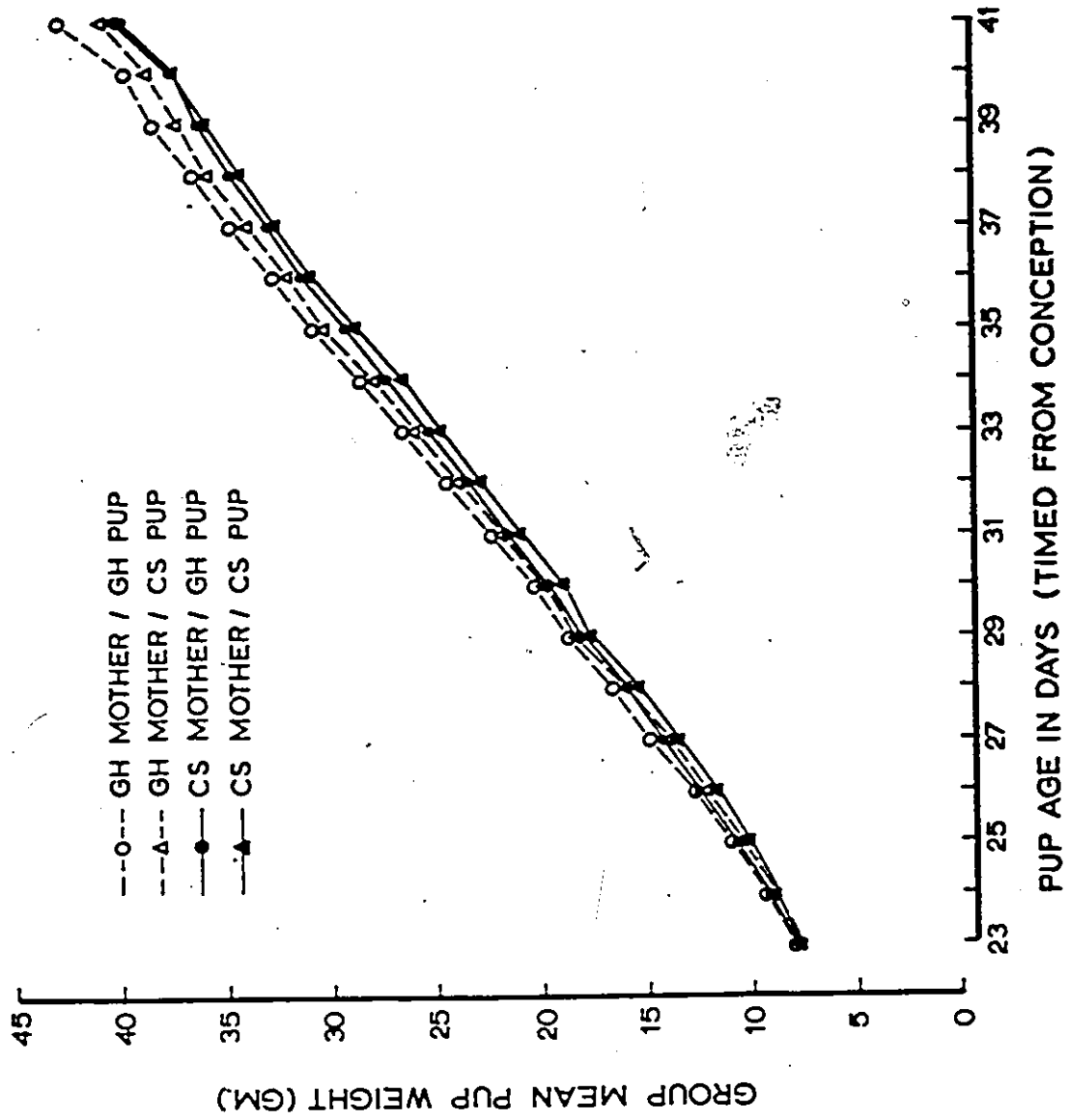
GROUP MEAN PUP WEIGHT (GM.)
45
40
35
30
25
20
15
10
5
0
23
25
27
29
31
33
35
37
39
41
PUP AGE IN DAYS (TIMED FROM CONCEPTION)
--o-- GH MOTHER / GH PUP
--Δ-- GH MOTHER / CS PUP
—●— CS MOTHER / GH PUP
—▲— CS MOTHER / CS PUP

Figure 42

Figure 43

Developmental parameters analysed by sex irrespective of prenatal or postnatal treatment. Females: open circles; males: filled circles.

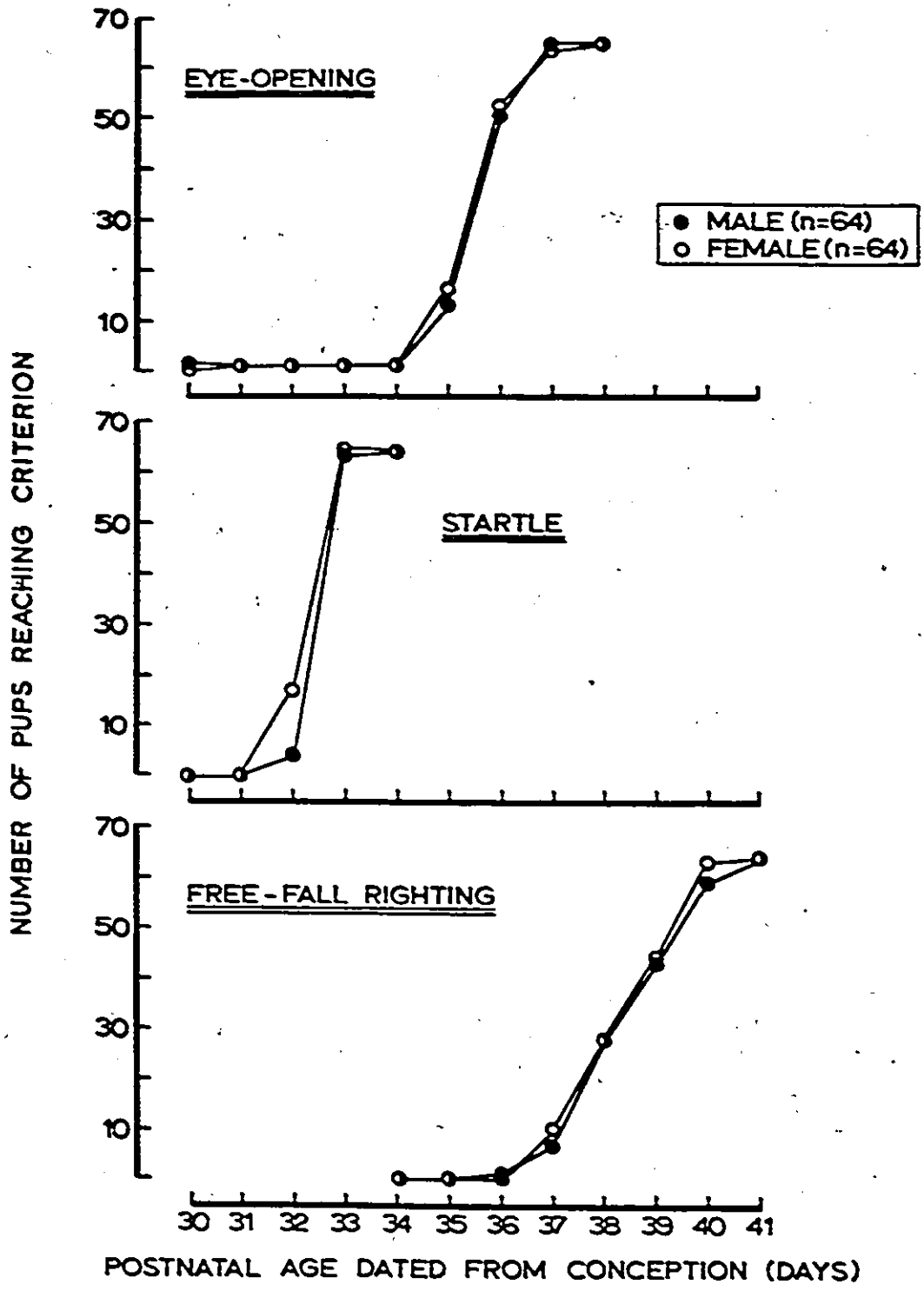
EYE-OPENING
STARTLE
FREE-FALL Rrighting
MALE (n=64)
FEMALE (n=64)
NUMBER OF PUPS REACHING CRITERION
70
50
30
10
30 31 32 33 34 35 36 37 38 39 40 41
POSTNATAL AGE DATED FROM CONCEPTION (DAYS)

Figure 43

CHAPTER IV

GENERAL SUMMARY AND CONCLUDING DISCUSSION

The experimental work reported here has been concerned with: (a) normal prenatal and postnatal developmental processes in the rat, and (b) the effects of growth hormone-treatment of the pregnant rat on these processes.

Summary of main findings

In Section I, four experiments were conducted in order to assess the structural development of the rat in early ontogenetic stages. The developmental course of body-weight, brain-weight, brain-cellularity, and brain-protein was observed through the prenatal, perinatal, and postnatal periods. While most of these parameters changed in a uniform and progressive fashion over time, the net increase in brain-cellularity as measured by DNA levels was biphasic. The discrete nature of the first phase of DNA accretion in the prenatal period afforded an opportunity to examine the claim that growth hormone, administered to the mother during pregnancy, resulted in hyperplasia of cells in the fetal brain (Zamenhof, 1942; Zamenhof, Mosley, and Schuller, 1966). The results of Experiments II, III, and IV, derived from assays on fetuses delivered by caesarean-section, established that the hormone treatment produced no detectable augmentation of fetal brain DNA, and

was similarly ineffective in enhancing placenta-weight, body-weight, brain-weight, or brain protein. It was also established that the treatment did not produce any effect on prenatal litter-size. The only noticeable outcome was a significantly greater increase in the body-weight of the pregnant rat. A discussion of these and other data in the general context of fetal growth regulation led to the conclusion that, with adequate nutrition, the fetus enjoys a relative autonomy from maternal influences, and that fetal development was not augmented through the continuous daily injections of growth hormone to the pregnant female.

These conclusions were difficult to reconcile with other reports of behavioural modification in the offspring of mothers who had received the hormone during pregnancy. If such differences could not be attributed to an acute influence of the hormone during the fetal period of development, particularly on the brain, then some other influence must be responsible for the effects that were observed. It was felt that the increase in maternal body-weight, which had been consistently observed in growth hormone-treated mothers may have been involved in the mediation of these postnatal behavioural modifications.

In Section II, the role of the mother in the determination of pup development was examined. In the first two experiments (Va and Vb), observations of maternal behaviour in the postpartum period suggested that some of the variability in maternal behaviour might be attributed to differences in maternal body-weight, and also to ambient conditions,

e.g., temperature. Further, it was observed that the amount of time the mother spent with her litter showed a substantial decline through the first two weeks postpartum. As this observation was in conflict with some reports on the behaviour of the lactating rat, maternal behaviour was subsequently studied in more detail in order to assess individual differences between lactating mothers more fully.

An automatic system was developed which permitted a 24 hr. record to be made of the amount of time the mother spent with her litter, together with a record of the frequency of her nest-periods (Experiment VI). The data obtained in this fashion accorded well with that of other workers who had employed a continuous recording system of different design (Grota and Ader, 1969). The amount of time mothers spent with their litters showed a progressive decline in the first two weeks postpartum. In addition, a circadian rhythm was also apparent: mothers invariably spent less time with their litters in the dark cycle than they did in the light cycle.

In Experiment VII, the influence of natural differences in maternal body-weight was studied. Using mothers in a wide range of body-weight, it was demonstrated that maternal body-weight did not affect gestation period, litter-size, or birth-weight of the offspring. Parturition invariably took place in the light period, and fundamental relationships were confirmed between litter-size and gestation period, and between litter-size and birth weight of offspring. In the postpartum period, a significant

correlation was found between total nest-time and maternal body-weight, as heavy mothers spent less time with their litters than did light mothers. A small but significant effect of foster mother on offspring development was found, and, females showed earlier maturation of the startle-reflex than males. Body and brain parameters of female offspring, autopsied at 56 days, tended to be inversely correlated with growth in the preweaning period. In the open-field in adulthood, activity of male offspring was positively correlated, and defecation negatively correlated, with amount of time spent with the mother in the first two post-partum weeks. The modulation of maternal behaviour was discussed, and a temperature-dependent model for nesting behaviour was proposed. Postnatal development and adult behaviour of offspring were interpreted from the theoretical standpoints of early experience and neoteny.

In Experiment VIII, a similar experimental design was employed to study the prenatal and postnatal effects of prenatally-administered growth hormone. Three different batches of growth hormone, administered daily from Days 7-20, resulted in greater increases in body-weight in the pregnant rat and in prolongation of gestation. The effect on gestation period was not due to a reduction in litter-size, neither was it due to absolute maternal body-weight at parturition. Several possibilities were suggested to account for the effect. In control mothers, the relationships between parturition and photoperiod, litter-size and birth-weight, and litter-size and gestation period were confirmed,

and birth-weight of male offspring was greater than that of females. In the hormone-treated mothers, the photoperiodicity of parturition was partially obscured, and no regression of litter-size on gestation period was apparent. The remaining relationships above were confirmed. No effect of the treatment on litter-size was apparent. Hormone-treated mothers spent less time with their foster-litters compared with control mothers, and nest-time was negatively correlated with maternal body-weight. When pup age was dated from parturition, offspring from growth hormone-treated mothers showed precocious maturation compared with offspring from control mothers. When pup age was dated from conception, the development of both groups was comparable. Thus, the previously reported developmental precocity of offspring from growth hormone-treated mothers could be accounted for by the prolongation of gestation that results from the treatment. No influence of foster mother on offspring development was apparent other than a slight effect on pup weight gain. No differences due to prenatal or postnatal treatments were apparent in females autopsied at 56 days, or in male adult behaviour in the open-field. Autopsy of mothers at Day 40 revealed that a significant growth response had occurred in the mothers treated with growth hormone, and, while liver growth appeared to be approximately proportional to the increases in body-weight, that of the kidneys was disproportional. The failure to confirm previous findings (Experiment VII) of an influence of foster

mother on postnatal development of offspring, and a relationship between pre-weaning experience and adult behaviour, was attributed to several factors: (1) prolongation of gestation in the hormone-treated group may have resulted in slight alterations in the postnatal development of offspring from these mothers, (2) the cross-fostering procedure resulted in temporal asynchrony in almost half of the mother-litter combinations studied, (3) possible ceiling-effects due to more early-handling, (4) possible qualitative differences in maternal body-weight between normally heavy mothers and those treated with growth hormone.

Some methodological considerations:--Jost and Picon (1970) have stressed the need for a precise estimation of fetal stages in developmental research and this consideration must, it seems, be extended to include the early stages of postnatal development. Developmental parameters are inextricably related to time, and errors of as little as 24 hr. may lead to substantially different interpretations of experimental results. For example, in Experiment VIII, it was possible to date offspring age both from time of conception and from time of parturition. It was shown that when pup age was based on time of conception pups from growth hormone-treated mothers showed no developmental precocity, whereas when pup age was dated from parturition these pups were precocious as Clendinnen and Eayrs (1961) and Ray and Hochhauser (1969) have reported. Similarly, it is evident that previous reports of fetal gigantism, and increments in brain structure claimed

to result from growth hormone-treatment (see review in Introduction) are open to serious question unless it can be demonstrated that age was precisely dated. In the absence of any such data, and in view of the results obtained here, it may be concluded that misdating of age could be responsible for these effects. The problem of dating clearly assumes more critical proportions when developmental parameters change rapidly in the space of a few hours, as does the perinatal population increase in fungiform papillae in the tongue (Henderson, 1973).

The dating problem is at the basis of another major difficulty, that of considering what is "normal." For example, normal parturition in the rat is said to occur between 20 and 22 days after conception, although little is presently known concerning the effects of slight prematurity, or postmaturity upon the subsequent development of the offspring. The combined data for control mothers in Experiments VII and VIII showed that over 90% of litters are born in the light cycle of the 21st day. For this particular strain of animals, under these conditions, that time must be considered "normal" and births that occur on Day 22 must be considered "abnormal." The question then becomes: How abnormal is a Day 22 birth? If this postmaturity is considered in terms of the total period of gestation, then an extra 24 hr in utero only amounts to an increase in time of approximately 5%. However, embryological and fetal growth is not linear from the time of implantation to the time of parturition, the fetus grows at an exponential rate over the last week of

gestation, and by Day 21 the placenta may be reaching the limits of its function. An extra day in utero at this stage, therefore, could be considered as a considerable relative increase in time. Postmaturity of several days in the rat is known to result in fetal mortality (see Barrow, 1970) but, to date, little is known about the effects of slight postmaturity, and only recently have attempts been made to examine the effects of prematurity (Konishi and Sun, 1972; Grota and Ambroso, 1973).

A similar problem in the definition of "normality" is encountered in the case of litter-size. In Section II, a litter-size of eight was selected for study in the postnatal period as the probability of mothers giving birth to at least this number was high, and a balanced sex-ratio could also be achieved. However, this number may not be ideal for the purposes of optimising postnatal effects that may be exerted by the mother. In addition, although postnatal litter-size has been experimentally varied in several studies (e.g., Sugita, 1918; Winick and Noble, 1966b; Edwardson and Eayrs, 1967; Dobbing and Sands, 1971), there is insufficient data available on the effects of litter-size in the normal situation; and what little is known has derived out of incidental observations (see Bond, 1958; Draper, 1968). In the study referred to earlier by Gainer (1974) mothers giving birth to litters in the range 3-14 were allowed to rear them normally, and observations on the development of the offspring were made. In general, indices of physical and CNS

maturation were found to be inversely related to litter-size, i. e., smaller litters showed earlier maturation. These data suggest that even though prenatal adjustments may occur in the mother so that the subsequent demands of the unborn litter may be adequately met in postnatal life (see Experiment VII), postnatal litter-size, under normal conditions is an important determinant of development.

Perhaps the key word here is adequate, and the question bears on the issue of what is "normal," what is "optimal," and where "maximal" lies with respect to the two (see earlier discussion, Experiment VII). These questions ultimately reduce to evolutionary considerations. For example, a small litter-size may have survival value in that the offspring will mature faster and therefore may more successfully protect themselves against noxious forces of the environment. Alternatively, the neoteny principle would suggest that precocity may impose a lower limit on developmental potential, and survival in later stages may be adversely affected. Thus, what may be construed as "deficits" in the early stages of development may, in effect, be essential transitory states of normal ontogeny (see Blaxter, 1961).

Although we are now more aware of the danger of anthropomorphic fallacy in the interpretation of animal behaviour (see Dobzhansky, 1956), a similar danger arises when extrapolations are made in the opposite direction (see Dobbing, 1973). Recent research has relied upon animal models to elucidate the normal and abnormal course of early

development, notably in the assessment of early nutritional influences. It must be stressed that the considerations above, particularly with regard to the principle of neoteny, are made within the restricted context of animal development. While neoteny may operate in some broad fashion in human phylogenetic evolution (Ashley-Montagu, 1955), and in individual ontogeny, the factors involved would presumably be of a more complex nature operating over a substantially longer period. Parenthetically, similar restrictions apply to considerations of the role of temperature in early development. Being ectothermic at birth, the rat is especially vulnerable to temperature changes, and theoretical implications (see Experiment VII) necessarily must be restricted to species which show a similar pattern of dependency upon an external source of temperature during the period of attainment of homoiothermia. Although ambient temperature changes may affect human neonatal behaviour (see Elder, 1970), they may ultimately prove less critical than in the rat, particularly as the CNS is at a later and less vulnerable stage of development at this time.

Another major methodological difficulty in studies of development concerns the quantification of observations. A specific example was discussed earlier (see Introduction) regarding the diversity of treatments grouped under the rubric of "early handling." The complex inter-relationships between mother, litter, and ambient conditions in early development (Plaut, 1970) necessitate more precise definition of the

independent variables, more detailed observation of the dependent variables, and evaluation of the possible role of intervening variables. In the case of the early ontogeny of the rat, the mother clearly plays a critical role, and can be viewed as a major intervening variable in studies involving experimental manipulation of the litter in the preweaning period. And yet, it is puzzling that studies on the effects of early experience have proceeded almost independently of the studies of maternal behaviour. Despite the publication twenty years ago of a paper by Seitz (1954) in which the early interrelationships between mother and litter were stressed, it was not until quite recently that these considerations have begun to be incorporated into the schema of developmental theory. Perhaps the reason for this stems from the fact that, whereas the studies on the effects of early experience have used a predominantly experimental orientation, maternal behaviour studies have been more ethological and naturalistic.

In studies of maternal behaviour over the last decade, however, there has been a noticeable trend towards a more parametric approach, particularly in the assessment of hormonal influences. The basic naturalistic conception of maternal behaviour has prevailed, however, in that such quantification as has been made was based on relatively short observation periods which, it now appears, were not representative. The approach taken by Grota and Ader (1969) was, in effect, no more than an extension of the quantitative orientation that studies of

maternal behaviour were beginning to take,[1] although the advantages inherent in using this method have not been widely accepted (see Moltz, 1971; Herbert, 1972; Lott, 1973; Adler, 1973).

The reason for this reception might be that the continuous recording technique has been viewed as an over-simplification of maternal behaviour, resulting in a resolution of a complex variable into a single discrete separation of the time the mother spends with the litter and the time she is absent from the nest. This criticism is justified insomuch as no observations are made on other aspects of maternal behaviour viz: retrieving, nest-building, grooming of young, et. It is, of course, important to establish whether other aspects of maternal behaviour decline in a similar fashion to nest-time in the first two postpartum weeks. It is interesting to note, however, that nest-time correlates well with the other criteria used to assess maternal behaviour (Grota and Ader, 1969). That the amount of time the mother spends with her litter is not a measure of nursing time per se is a reasonable objection. Further work is required to establish what proportion of nest-time is constituted by nursing-time, and whether this proportion is constant through lactation. However, the measure of nest-time, and the frequency and patterning of nest-periods, remain important features of maternal behaviour. Whether the mother is nursing or not, her presence in the nest engenders a markedly different set of conditions from when she is not there, conditions

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[1]The first attempt to monitor maternal behaviour continuously appears to have been made by Gustaffson (1948) - see Experiment VI.

that may be critical to the development of the young.

It must be emphasised that early experience of the litter and maternal behaviour are inextricably related and it becomes necessary (especially in view of the possibility that adult behaviour may be determined by preweaning experience (Experiment VII), and that brain cell-differentiation may be similarly influenced - see below) that the two be considered together. The amount of time the mother spends with her litter and the patterning of her nest-periods are the only reliable parametric indices presently available, by which preweaning experience of the litter may be assessed. The investigation of maternal behaviour as a determinant of early experience, and therefore as a major epigenetic source of influence on development, should prove extremely profitable in future studies.

With the use of a continuous recording system of different design (Experiment VI), the results obtained by Grota and Ader (1969) have been confirmed, and suggestive evidence has been brought forward linking preweaning experience to adult behaviour (Experiment VII). In the final part of this discussion, an attempt will be made to integrate the results of studies on the adult behaviour of offspring from growth hormone-treated mothers with the present findings.

In the Introduction, several studies were reviewed which suggested that the offspring from growth hormone-treated mothers showed behavioural superiority in adulthood. The first point that should be

made, perhaps, is that "behavioural superiority" or "intelligence" are both enigmatic terms when applied to animals. In the study by Block and Essman (1965), for example, extinction behaviour was assessed following a single exposure to electric shock. The criterion for extinction was met if a subject entered a chamber where it first received shock. Over 20 extinction trials, offspring from growth hormone-treated mothers showed a more rapid extinction of the avoidance response than did control offspring. It was reasoned that as the GH offspring had more rapidly extinguished what was no longer an appropriate response, they had demonstrated adaptive behaviour. Alternatively, it may be argued that willingness to re-enter a situation in which a strongly aversive experience had occurred was maladaptive, or indicative of poor memory. A more circumspect conclusion might be that one group of subjects behaved differently from the other.

Clendinnen and Eayrs (1961) used a more traditional assessment of "intelligence," the Rabinowitz and Roswold (1951) modification of the Hebb-Williams maze. Subjects were thirst-motivated to run from a starting area through a maze to a goal box. Twelve different mazes were used and deviations from a direct course were scored as errors. "Intellectual capacity" was indexed by the total error score over the twelve problems. Offspring from GH mothers made significantly fewer errors than control offspring, and it was concluded that the GH-offspring had demonstrated enhancement in the performance of cortically-mediated

behaviour. Again, there is the problem of interpreting exactly what these differences in behaviour signify. High error scores in the Hebb-Williams maze have been attributed to a greater tendency to explore (Woods, 1959), and others have questioned the use of this test on the grounds that performance is dependent on visual cues (Warren, 1965). There is an additional problem in the study above, in that GH-offspring may have been heavier at the time of testing and there is no guarantee that water-deprived animals of differing body-weight experience the same degree of thirst motivation.

In a study by Ray and Hochhauser (1969), offspring from GH-treated or control mothers were reared in either enriched or isolated environments following weaning. Differences were found in adulthood only between those groups reared in the isolated condition, The main difference in performance was observed in the Lashley III maze. In this procedure, the food-deprived subject runs through a maze to a goal-box. Performance is assessed as the number of errorless trials to a given criterion. Enhanced performance of GH offspring was also found in a shuttle-box avoidance task in which, following the onset of a discriminative stimulus (light) the subject is allowed 10 sec to move to a dark compartment to avoid shock.

In addition to the difficulties already mentioned in interpreting such behavioural changes, the absence of any changes in the enriched condition is difficult to explain. It may be, as the authors suggest,

that a ceiling effect occurred and this seems reasonable from the fact that, in general, subjects reared in the enriched condition performed better in the learning tasks than did those reared in the isolated condition. Alternatively, it might be argued that if neuronal hyperplasia had resulted from the hormone treatment, a further augmentation of brain structure should have occurred following experience in the enriched environment as this treatment is reported to increase the ratio of glia to neurons (Diamond, Krech and Rosenzweig, 1964).

In summary, it seems clear that all of the above studies[1] provide evidence of a difference in adult behaviour between GH-offspring and control offspring. However, there are some overriding problems in interpreting the results. First, there is no indication that litter effects (King, 1969; Abbey and Howard, 1973) were taken into account in statistical treatment of the data. Second, in none of the studies was a fostering procedure used, and it is therefore impossible to rule out a postnatal mediation in the effects observed. In the study reported in the appendix (A), cross-fostering was conducted and only one representative from a litter was tested. In terms of "performance" control offspring reared by GH-mothers were superior to the GH-offspring reared by control mothers, although, again, the effects could have been due to differential motivation and factors unrelated to higher brain function. The failure to find any systematic differences in adult behaviour in Experiment VIII of the present work has already been discussed in terms

[1]Also see Sara and Lazarus (1974).

of a disruption in the synchronous relationship between mother and litter in the postpartum period. The increased amount of early handling may have also reduced the likelihood of detecting differences in adult behaviour. An additional possibility is that the extremely uniform conditions of rearing may have suppressed qualitative differences between GH mothers and control mothers.

Having considered some of the methodological issues in this area, we may now turn to the explicit suggestion that differences in adult behaviour can be attributed to modifications of cortical structure (see Introduction). The argument has been advanced that this modification is established under the acute influence of growth hormone in the prenatal period, and takes the form of neuronal hyperplasia. Thus, it is proposed that a relatively slight increase in the total number of cells in the brain finds expression in a modification of adult behaviour. The evidence from other studies (Eayrs, 1961; Altman, 1967) would suggest instead that if any broad relationships were to be established between CNS structure and function at this level, it would be the pattern of cell-connectivity (and therefore cell-differentiation) that was involved rather than any change in the absolute number of cells. In evolutionary terms, it is not the density of cells that has been associated with higher intellectual function so much as an increase in the elaboration of the neuropil reflected as an increase in the cell-gray coefficient (see Bekoff and Fox, 1972).

Cortical cells are little differentiated at birth in the rat. For the sensori-motor cortex, Eayrs and Goodhead (1959) found that axonal density increased most rapidly between postnatal Days 6-18, and dendritic density between Days 18-24. Previous attempts to quantify developmental changes in neuronal connectivity have been made largely on the basis of such indirect measures as the cell-gray coefficient, and the density of axons, dendrites, and dendritic spines (see Aghajanian and Bloom, 1967; Bekoff and Fox, 1972). More recently, however, direct observations have been made on the ultrastructural development of morphologic junctions between cells. Aghajanian and Bloom (1967) found a rapid increase in synaptic junctions in the parietal cortex in the third postnatal week, and Bloom (1972) has reported an earlier, triphasic (cf. Del Cerro and Snider, 1968), and more protracted period of synaptogenesis in the cerebellar cortex in the postnatal period. This suggests then that if any changes in cell-differentiation and connectivity were to be established the optimum time would be during the first three postnatal weeks, i.e., at the preweaning stage.

To date, little work has been done on the important subject of what factors influence the process of synaptogenesis. Bunge, Bunge, and Petersen (1967) have emphasised inherent genetic determinants of the process, having observed a substantial development of synapses in explanted fetal cord tissue (afferent and efferent input abolished). It is hard to imagine, however, that synaptogenesis completely escapes

environmental influence. A reduction in the number of dendritic spines in the visual cortex has been reported in dark-reared mice (Valverde, 1967) which appears to be permanent (Ruiz-Marcos and Valverde, 1969), and as the majority of cortical synapses are established on the dendritic spines (see Colonnier, 1968) it would be expected that corresponding deficits would also have arisen in the ontogeny of synapses. That the structural and functional properties of immature neurons differ from those of mature neurons (see Purpura, 1972) suggests further that early-induced changes in synaptogenesis might subsequently give rise to profound and long-lasting modifications in CNS organisation.

In the study by Clendinnen and Eayrs (1961) several parameters of cortical neuron structure were compared in the offspring of growth hormone-treated and control mothers. No histological evidence of hyperplasia was found although compared with controls, growth hormone offspring showed a 20% increase in the cell-gray coefficient, a 23% increase in the number of dendrites associated with each neuron, and a 22% increase in the average length of dendrites. These structural increments were estimated to have increased substantially the probability of interaction between neurons. However, the authors found it difficult to reconcile the fact that growth hormone, administered prenatally, had influenced cortical cell-differentiation which occurs chiefly in the postnatal period. The possibility was considered that cortical cell-differentiation was advanced in the growth hormone offspring. The

finding of an increase in birth-weight and a progressive widening of the body-weight difference with controls in the postnatal period was adduced in support of this contention.

The experiments reported in Section I, however, firmly establish that there is no advance in body or brain growth in fetuses of growth hormone-treated mothers, and any difference in birth-weight can be accounted for by extended gestation (Experiment VIII). Further, growth hormone offspring fostered to control mothers do not grow faster than control offspring fostered to growth hormone mothers, rather the converse appears to be the case (Experiment VIII, Apendix A). Unless growth hormone establishes a higher capability of protein synthesis prenatally which is subsequently expressed in a greater degree of cytodifferentiation postnatally (see Jacobson, 1970), the logical conclusion of these data must be that the neuronal hypertrophy observed by Clendinnen and Eayrs (1961) was established during the normal postnatal period of cell-differentiation under the influence of the growth hormone-treated mother whose behaviour is shown here (Experiment VIII) to differ from that of the control mother.

The environmental dependency of the developing nervous system has been well established (see Bekoff and Fox, 1972) and hypotheses have been advanced to account for the effects of early experience on cortical development (Schapiro and Vukovich, 1970). The conclusions above raise the challenging possibility that the early plasticity of the developing

nervous system of the rat is critically influenced by the relationship between mother and litter in the preweaning period, and through this dependency modifications in the structural and functional organisation of the brain may be established.[1]

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[1] In a recent publication (Sara and Lazarus, 1974) the claim is again made that the prenatal treatment with growth hormone (porcine) results in a permanent increase in the number of cortical neurons and in an "enhancement of learning ability." It is possible that porcine growth hormone differs, in effect, from bovine and ovine preparations, although what evidence there is (Angervall and Lundin, 1962) argues against this. In view of the results obtained in the present work, it seems more probable that imprecise dating of pup age (arising from extended gestation - see Experiments VIIIa and VIIIb) together with postnatal maternal influences (see Experiments VII and VIII) may have been responsible for the structural changes observed, and that postnatal maternal influences were responsible for the differences in adult behaviour (see also General Summary and Concluding Discussion).

A discussion of the above paper together with a related report (Sara, V. R. Lazarus, L., Stuart, M. C., and King, T. (1974)). Fetal brain growth: selective action by growth hormone. Science, 186:446-447) is given in Croskerry, P. G., and Smith, G. K. (1975)). Prolongation of gestation by growth hormone: a confounding factor in the assessment of its prenatal action. Science (in press).

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APPENDICES

Appendix A

DISCRIMINATED AVOIDANCE BEHAVIOUR OF OFFSPRING FROM CONTROL AND GROWTH HORMONE-TREATED MOTHERS

Previous reports have suggested that the offspring from mothers treated with growth hormone during pregnancy show behavioural superiority in adulthood (see Introduction), although such an interpretation is open to question on the basis of the behavioural measures employed (see Final Discussion and Conclusions) and the statistical treatment of the data obtained (see King, 1969; Abbey and Howard, 1973). Another major difficulty in interpreting these studies is that, in all cases, the offspring from growth hormone-treated mothers were reared by these same mothers and the possibility of postnatal mediation may not therefore be excluded. The purpose of the present study was to investigate the adult behaviour of GH-offspring who were not exposed to the postnatal influence of the GH-mother, and of control offspring who were.

The behavioural measure used was discriminated avoidance from electric shock. This task was selected for several reasons. First, the difficulty which normal rats have in successfully performing discriminated avoidance (Meyer, Cho, and Weseman, 1960) would presumably facilitate the detection of "behavioural superiority" in growth hormone-treated rats (*c.f.*, Warden, Ross, and Zamenhof, 1942). The difficulty of the task would also give rise to a protracted period of acquisition which would be useful for comparing individual performances. Second, although this measure is time-consuming and therefore permits only few subjects to be tested, behaviour in the operant situation may be more precisely measured.

Hooded rats from the McMaster colony were used. Primiparous females in the weight range 170-190 gm. were mated and insemination confirmed by vaginal lavage. Pregnant females were randomly assigned to one of two groups. The growth hormone group (GH) received daily subcutaneous injections of growth hormone (NIH-GH-B14) contained in 0.2 ml. physiological saline in the pH range 8.0-9.0, from the seventh to the twentieth day of gestation. The control saline group (CS) received the same volume of the vehicle by the same route. Animals were injected and weighed around midday. Cages were inspected for litters in the morning, at midday, and in the evening. Within twelve hours of parturition, litters were weighed, culled to ten pups, and GH-pups were fostered

to CS-mothers and vice versa (see Table XXXIII. Beginning on Day 6, pups were sampled from both groups for use in another project; the extent and rate of litter reduction was comparable in the two groups. Litter weights were recorded daily to Day 15. Group mean pup weights are shown in Figure 44. No statistically significant differences between the groups were found on Day 2, but by Day 5 CS-pups were significantly heavier (two-tail t-test: $p < 0.01$). CS-pups remained significantly heavier until Day 10, after which time the difference in group means was not statistically significant. Maternal body-weight during pregnancy and over the first 15 days postpartum is shown in Figure 45. By prenatal Day 13 the GH group was significantly heavier than the CS group ($p < 0.05$) and remained so during most of the postpartum period.

In adulthood, males were randomly selected (one per litter) for testing in an operant paradigm involving discriminated avoidance from electric shock. A blind procedure was first used (the experimenter did not know which group the animal was from) to shape subjects to lever-press to escape continuous foot-shock. Five seconds prior to shock onset a warning signal (S^D) consisting of two lights and a tone came on. The lights were mounted to the side of and slightly above the lever on the functional panel. The box was contained in a sound-proof chest in a darkened room. Throughout the first shaping session, shock intensity was 0.5 mamp. and the inter-trial interval (ITI) was 30 sec. Where shaping was difficult the shock level was increased to a maximum of 2.0 mamp. and the ITI decreased to a minimum of 15 sec. Most subjects made their first lever-press within 10 min. and reached a criterion of 10 consecutive escape responses in 30-50 min. When criterion was reached, the apparatus was switched to automatic operation for 30 min. with continuous shock level at 0.5 mamp. and ITI of 30 sec. On Day 2, subjects were allowed 10 continuous shocks at 0.5 mamp followed by pulsed shock at 0.8 mamp. for one hour. On subsequent days; subjects were run for one hour with pulsed shock at 0.5 mamp.

The results over seventeen daily one-hour sessions are shown in Figure 46. Discriminated avoidance was calculated by expressing the total number of responses during the five seconds the S^D was on as a percentage of the total number of S^Ds presented. Each of the control subjects (reared by GH-mothers) demonstrated discriminated avoidance behaviour. Following acquisition over nine sessions, the group average was maintained at about 70-80%. In contrast, the percentage of discriminated avoidance responses made by the GH subjects (reared by control mothers) rarely exceeded chance level over the seventeen sessions. Two other adult males from untreated, non-fostered control litters (CC) also showed no acquisition of discrimination behaviour.

Clearly, any interpretation of these results must be limited in view of the small sample of animals that was used. However, by using only one subject from a litter, statistical independence is achieved and the absence of any overlap in the results suggests a real difference between the groups. The design of the experiment does not allow any conclusions to be drawn regarding the origin of this difference. Ideally, a split-fostering design should be used in which mothers reared half of their own litter and half of the litter from a mother in the other group. This would allow some partitioning of prenatal and postnatal influences. For equality in all groups, however, it might be necessary to deliver pups be caesarean-section in order to eliminate any effects of extended gestation (see Experiment VIII) in the GH group.

Summary

Prenatal treatment with growth hormone over the last two-thirds of gestation resulted in a significant increase in maternal body-weight which was sustained, in part, in the postnatal period. GH-offspring reared by control mothers showed a lower rate of body-weight gain in the first two postnatal weeks. In adulthood, GH males did not show discriminated avoidance behaviour whereas control males reared by GH-mothers did.

Figure 44

Mean weights in first two weeks of pups born to control mothers and reared by growth hormone-treated mothers (---▲--) and pups born to growth-hormone mothers and reared by control mothers (—●—). Pup age is dated with respect to the onset of pregnancy, i.e., prenatal Day 21 ≡ postnatal Day 0.

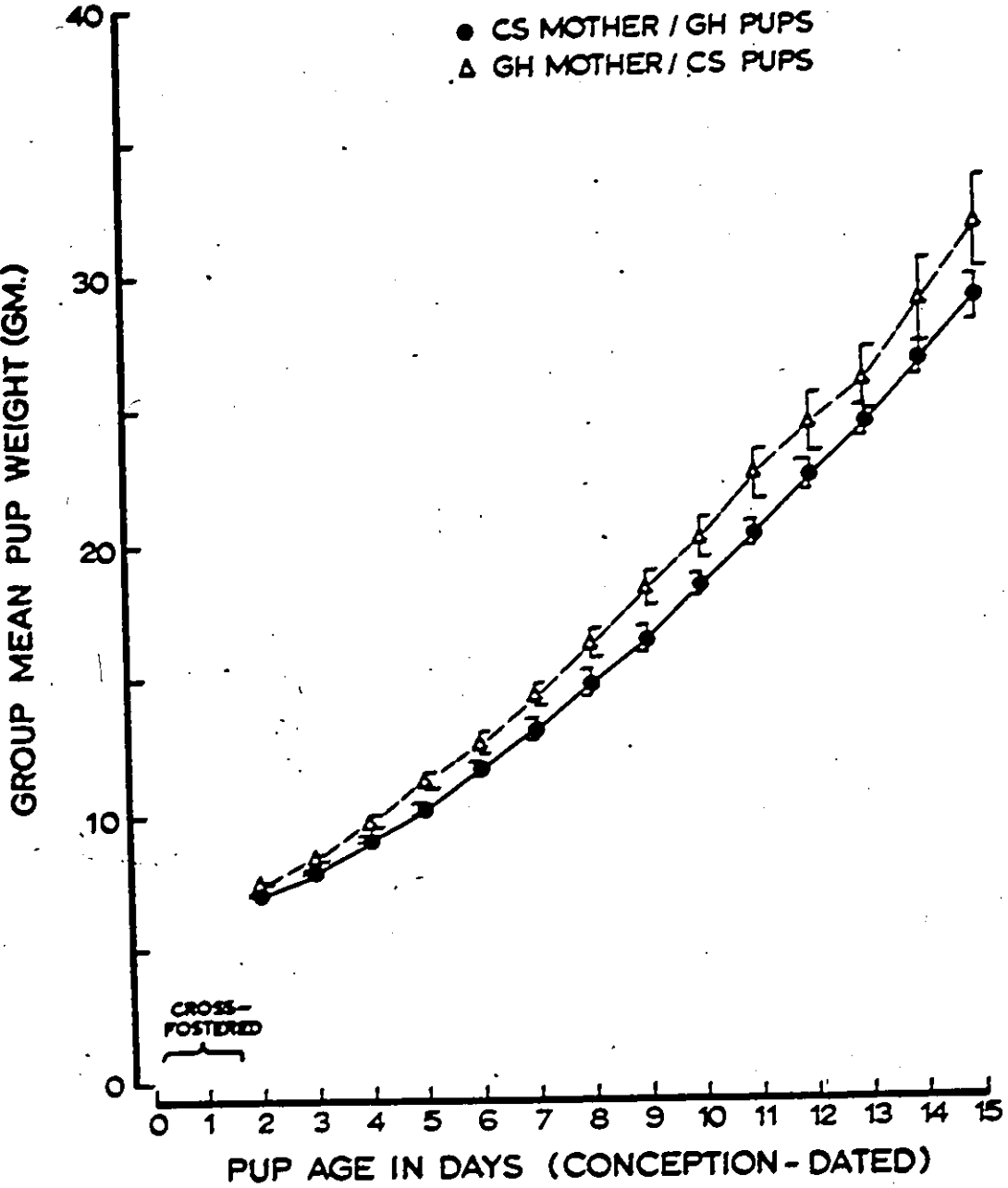
● CS MOTHER / GH PUPS
△ GH MOTHER / CS PUPS
GROUP MEAN PUP WEIGHT (GM.)
40
30
20
10
0
CROSS-FOSTERED
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
PUP AGE IN DAYS (CONCEPTION - DATED)

Figure 44

Figure 45

Body-weight gain in pregnancy and in the first two weeks postpartum of six animals receiving 3 mg. of growth hormone (NIH-GH-B14) daily from the seventh to the twentieth day of gestation (filled circles), and of six saline-injected controls (open circles). Postnatally, standard errors of means represented by shaded area.

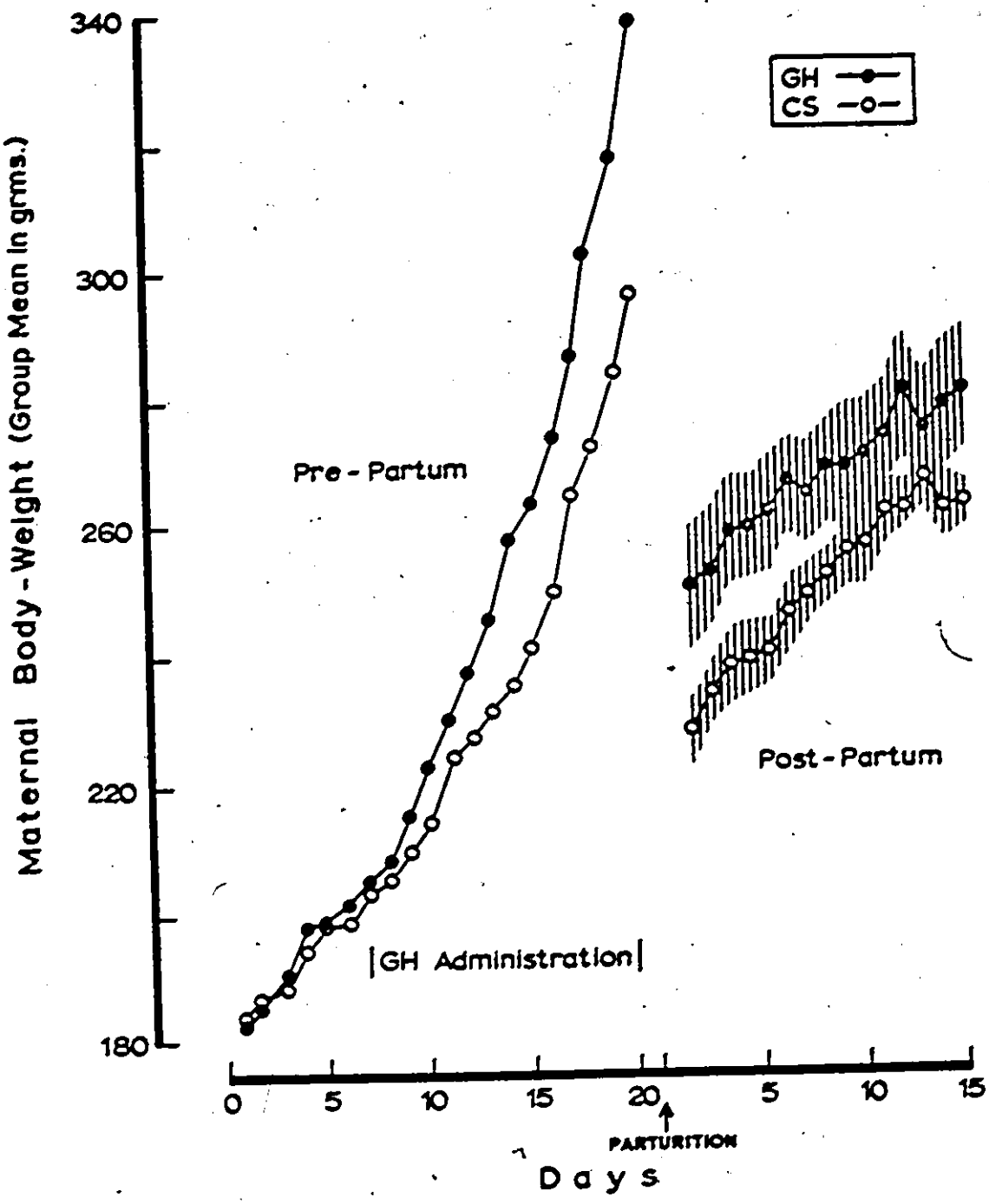
340
300
260
220
180
Maternal Body-Weight (Group Mean in gms.)
GH
CS
Pre-Partum
Post-Partum
GH Administration
0
5
10
15
20
5
10
15
PARTURITION
Days

Figure 45

Figure 46

Precent discriminated avoidance in individual adult males (top eight plots). Controls reared by growth-hormone-treated mothers are shown on left (E_2, J_1, M_1), growth-hormone offspring reared by control mothers in middle (B_2, F_1, C_2), and untreated, non-fostered controls on right (T_1, U_1) Group data shown below. Broken line in C_2 plot indicates apparatus failure in one session.

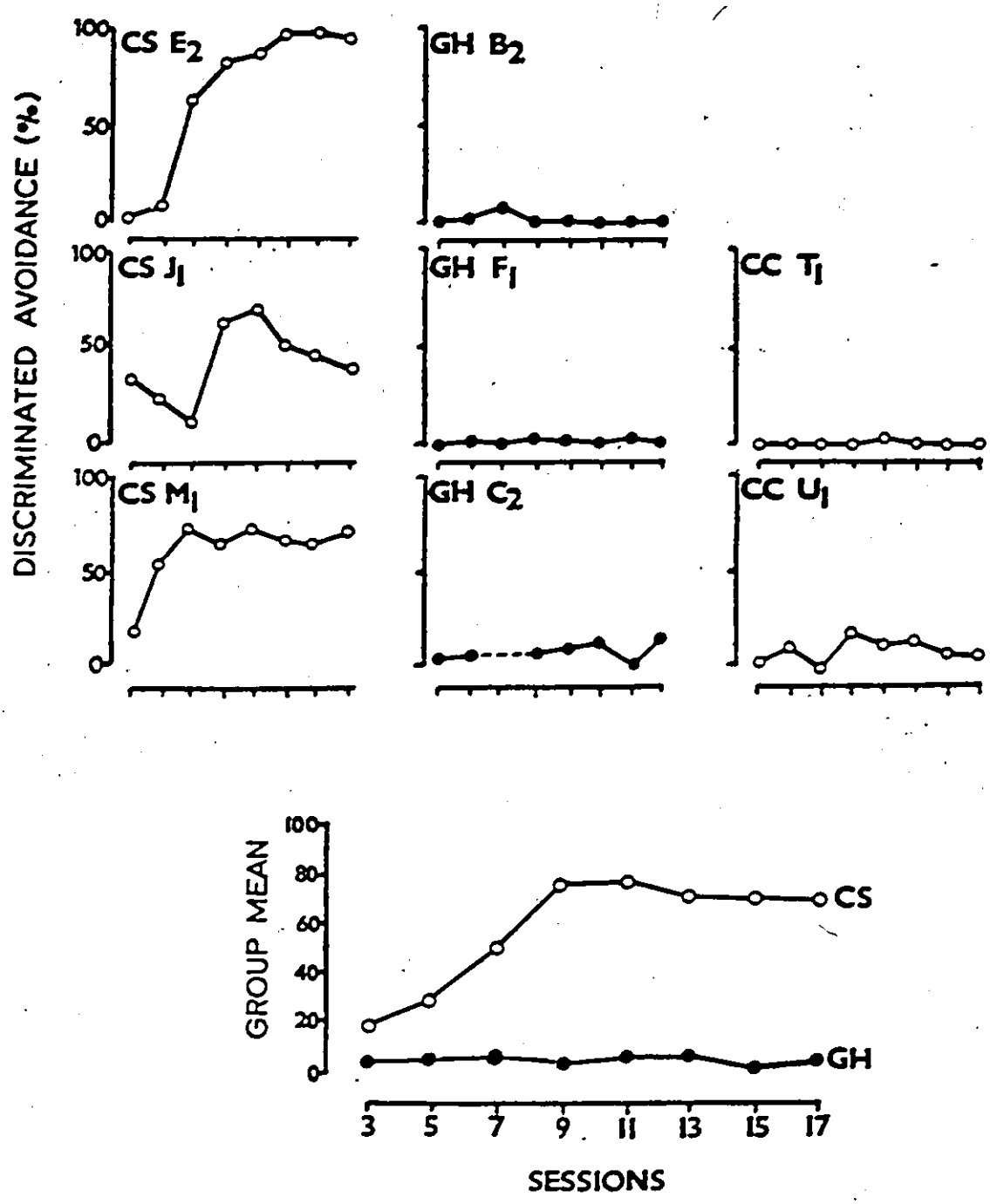
DISCRIMINATED AVOIDANCE (%)
CS E2
GH B2
CS J1
GH F1
CC T1
CS M1
GH C2
CC U1
100
50
0
GROUP MEAN
80
60
40
20
CS
GH
3
5
7
9
11
13
15
17
SESSIONS

Figure 46

Table XXXIII

Litter-size, weight, and approximate time of parturition in growth hormone and control groups.

Table XXXIII

Group	Mother	Time of Parturition	Number in Litter	Litter Weight (gm)	Average Pup Weight (gm)
GH	B	OVERNIGHT 21-22	15	82	5.466
	C	MIDDAY 21	14	81	5.786
	S	MORNING 22	13	80	6.154
	F	MORNING 21	13	72	5.539
	D	OVERNIGHT 21-22	11	73	6.636
	O	EVENING 21	11	63	5.727
			12.83		5.884
CS	E	MIDDAY 22	12	63	5.250
	J	OVERNIGHT 21-22	10	65	6.500
	N	MIDDAY 21	13	70	5.384
	G	MORNING 21	11	56	5.090
	Q	MIDDAY 21	12	67	5.583
	M	EVENING 21	10	59	5.900
			11.33*		5.618*

*not significantly different from GH value

Appendix B

PUP BODY-WEIGHT ON POSTNATAL DAY 15
(Experiment VII)

Analysis of Variance

Source of Variation	df	Sum of Squares	Mean Square	F	p
Total	127	1158.6	-	-	-
Prenatal (Pre)	1	18.8	18.8	8.0	<.01
Postnatal(Post)	1	148.8	148.8	63.5	<.01
Sex	1	78.1	78.1	33.3	<.01
Pre x Post	1	116.3	116.3	49.6	<.01
Pre x Sex	1	6.1	6.1	2.6	ns
Post x Sex	1	0.6	0.6	0.3	ns
Post x Pre x Sex	1	0.9	0.9	0.4	ns
Litter Effect	24	562.5	23.4	10.0	<.01
Residual	96	224.9	2.3	-	-