THE DEVELOPMENT OF THYROID AND ADRENAL FUNCTION IN FETAL AND NEWBORN GUINEA PIGS

A thesis presented for the degree of Doctor of Philosophy

BY

ALI FADEL ALWAN

UNIVERSITY OF SOUTHAMPTON

FACULTY OF SCIENCE

March 1987

TO MY DEAR WIFE JANNAN

LOVELY DAUGHTER NOOR and

BEAUTIFUL SON MOHAMMED

ACKNOWLEDGEMENTS

My Thanks to Professor K.A. Munday, Dr B.J. Parsons and Professor N.R. Saunders the three Heads of Department during my study for the provision of excellent research facilities in the Department of Physiology and Pharmacology.

I wish to express my sincere gratitude and deep appreciation to my supervisor, Dr. A.L. Thomas for his time and continuous supervision, guidance, suggestions and encouragement throughout the period of my graduate study. My thanks go to Dr. A. Noble and Dr. E. Thomas for their help and advice, and to the animal house staff for the care of my animals.

Thanks with my best regards also go to my parents for their constant support and encouragement throughout my education. Finally my warm thanks to my father in law Mr. Hussan Alwan Albaghdady for his valuable support throughout my Ph.D. study.

University of Southampton

FACULTY OF SCIENCE

PHYSIOLOGY AND PHARMACOLOGY

Doctor of Philosophy

THE DEVELOPMENT OF THYROID AND ADRENAL FUNCTION IN FETAL AND NEWBORN GUINEA PIGS

by Ali Fadel Alwan

ABSTRACT

There was a linear increase in fetal body weight from 27 days to full term. The thyroid gland of the fetal guinea pig is relatively large, follicles are present at 35 days following conception and progressively increase in number and size with increasing fetal age, colloid content increases proportionately. Thyroxine (T4) and Triiodothyronine (T3) are present in fetal plasma. Fetal plasma T4 concentration was greater than that of the mother between 50 and 62 days of gestation while fetal plasma T3 concentration was less than that of the mother throughout gestation.

Fetal adrenal gland activity increased towards the end of gestation. The plasma cortisol levels measured in pregnant guinea pigs after anaesthesia were higher than those in non-anaesthetised animals. In the fetuses this difference was only seen within 3 to 4 days of birth.

The pituitary in the fetus displayed signs of cytological differentiation at the beginning of the second half of intrauterine life. After 60 days until full term the pituitary gland showed a rapid increase in weight and an increase in the number and size of basophil and acidophil cells.

After birth neonatal plasma T4,T3 and cortisol levels increased rapidly to reach their highest values at about 24 hours. The rate of plasma T4 concentration decline in neonatal animals was 27.7 hours while the plasma T4 half-life in adult male guinea pigs was 23.4 hours.

Blocking the secretion of thyroid hormones by administering PTU to pregnant guinea pigs resulted in massive growth of the fetal thyroid and impairment of bone development. The maternal thyroid was little affected by the treatment. In these animals there was a clear decrease in fetal plasma T4, T3 and cortisol concentrations at 50 and 60 days of intrauterine life as well as in the plasma of one hour old guinea pigs.

Daily adminstration of dexamethasone (10 mg) from 55 days of pregnancy for 6 days resulted in a depression of fetal body and organ weight and a depression of maternal and fetal plasma T4, T3 and cortisol concentrations but an increase in the fetal plasma T3/T4 concentration ratio suggesting enhanced β deiodination of T4.

Maternal thyroidectomy at 39 days of gestation resulted in a decrease in fetal plasma T4, T3 and maternal T4 concentrations when measured at 60 days of gestation. No changes were observed in maternal plasma T3 or cortisol or in fetal plasma cortisol. Large thyroid follicles and areas of papillary infolding of the columnar epithelium were seen in the thyroid glands of the fetuses from thyroidectomised mothers.

CONTENTS Page

RESULTS

ILLUSTRATIONS

Figures R2 - 2, 3, 4, 5, and 6 thyroid of 35, 35, 38, 55, 60 day old fetuses, respectively and Fig. R2-7 thyroid of neonatal guinea pig from untreated mother.

Figures R2 - 8 and 9 adrenal of 35 and 50 day old guinea pig fetuses from untreated mother.

Figures R8 - 13 and 14 bone of 24h old neonatal guinea pig from untreated mother.

Figures R8 - 3, 4, 5, 6 and 7 thyroid of 30, 30, 35, 50 and 50 day old guinea pig fetuses respectively and Figures R8- 8 and 9 thyroid of 24 hour old neonatal guinea pig all from PTU treated mothers.

Figures R8 - 10 and 11 anterior pituitary of 50 and 60 day old guinea pig fetuses from PTU treated mothers.

Figure R8 - 12 skin of a 30 day old guinea pig fetus from a PTU treated mother.

Figure R8 - 15 bone of a neonatal guinea pig from a PTU treated mother.

INTRODUCTION

INTRODUCTION

The Adrenal Gland

All mammals have fetal adrenal glands comprised of cortical and medullary regions which have different embryological origins and thus form two separate functional and morphological structures within a single capsule. When developed both glands are usually ovoid structures positioned at the root of, and cranial to, the renal vessels. In guinea pigs the adrenal cortical cells begin to group together at 22 days and the first detectable migration of medullary cells into the cortex occurs about four days later. Steroid synthetic activity is present in adrenals of 28 day old fetuses and this activity increases with age. Thus the fetal adrenal cortex is capable of producing androgens at 22 days whilst the adrenal medulla secretes hormones as early as 30 days of gestation (Price and Ortiz, 1965). In human fetuses C-19 steroids and cortisol are first detected in the gland after 16 weeks (Block, Benirschke and Rosemberg, 1956) In rats corticosteroids are first detected at about 13 days (Josimovich, Ladman and Deane, 1954). Jost and Picon (1970) suggested that the first appearance of adrenal tissue and the biochemical capability of synthesising hormones were independent of a functioning pituitary but that the secretion of hormones into the blood required an intact pituitary. Lack of an intact fetal pituitary leads to a reduction in adrenal size and atrophy of the cortical tissue in the following species, human (Brewer, 1957), sheep (Liggins and Kennedy, 1968), rat (Wells, 1947) and mouse (Eguchi, 1961).

endocrine gland

The adrenal is one of the largest in the fetal guinea pig. The fetal adrenal cortex in most mammals develops an inner zone of cells. In humans the fetal adrenal is extremely large because of the considerable development of this internal zone the "fetal zone" which regresses after birth. The outer zones which make up most of the postnatal cortex is called 'adult cortex' (Anderson and Winter, 1985). Ross (1967) suggested that the fetal adrenals may produce steroids

before zonation. In guinea pigs the adrenal blastema the initial inner and outer zones of the adrenal cortex, develops and becomes recognised at 21 to 22 days when the cortex is found to contain androgens. By mid gestation when zonation of the adrenal is apparent, enzymes important in steroid biosynthesis can be demonstrated in cortical cells by histochemical and biochemical techniques (Bloch, 1969).

The adrenal cortex comprises 80% of the weight and volume of the adult human adrenal. Gross examination of the guinea pig adrenal shows the cortex to be composed of an outer yellow and an inner brown region. These zones represent 33% and 66% of the adult adrenocortical volume respectively (Strott, Goff and Lyons, 1981). Guinea pig adrenal cortex produces aldosterone and glucocorticoids from different cells and each cell type is controlled by a specific regulator. The glomerulosa secretes aldosterone. It is generally accepted that the cells of the zona fasciculata produce and secrete cortisol and that the zona reticularis cells probably do not. Both zones respond to adrenocorticotrophic hormone (ACTH) and as a result of the low enzymatic activity of the zona reticularis it is suggested that in the adult guinea pig it has functions usually attributed to the liver i.e. steroid catabolism rather than synthesis (Martin and Black, 1982). There is a very rich blood supply to the mammalian adrenal with branches coming from the aorta, renal and lumbar arteries. The full function of the cortex is dependent on a high arterial blood supply (Harrison and Patterson, 1980).

Control of steroid secretion

The adrenal steroids are synthesised from cholesterol which the adrenal cortex is able to take up from the blood. Blood cholesterol can be derived either from the diet or from liver biosynthesis. Cholesterol is stored in the cortex in a free or an esterified form. The more important enzymes involved in the synthesis of the two major glucocorticoids, cortisol and corticosterone, are shown in figure 1:1 The adult in all mammalian species can carry out all the enzyme steps shown. In contrast the activity of these enzymes may not be fully

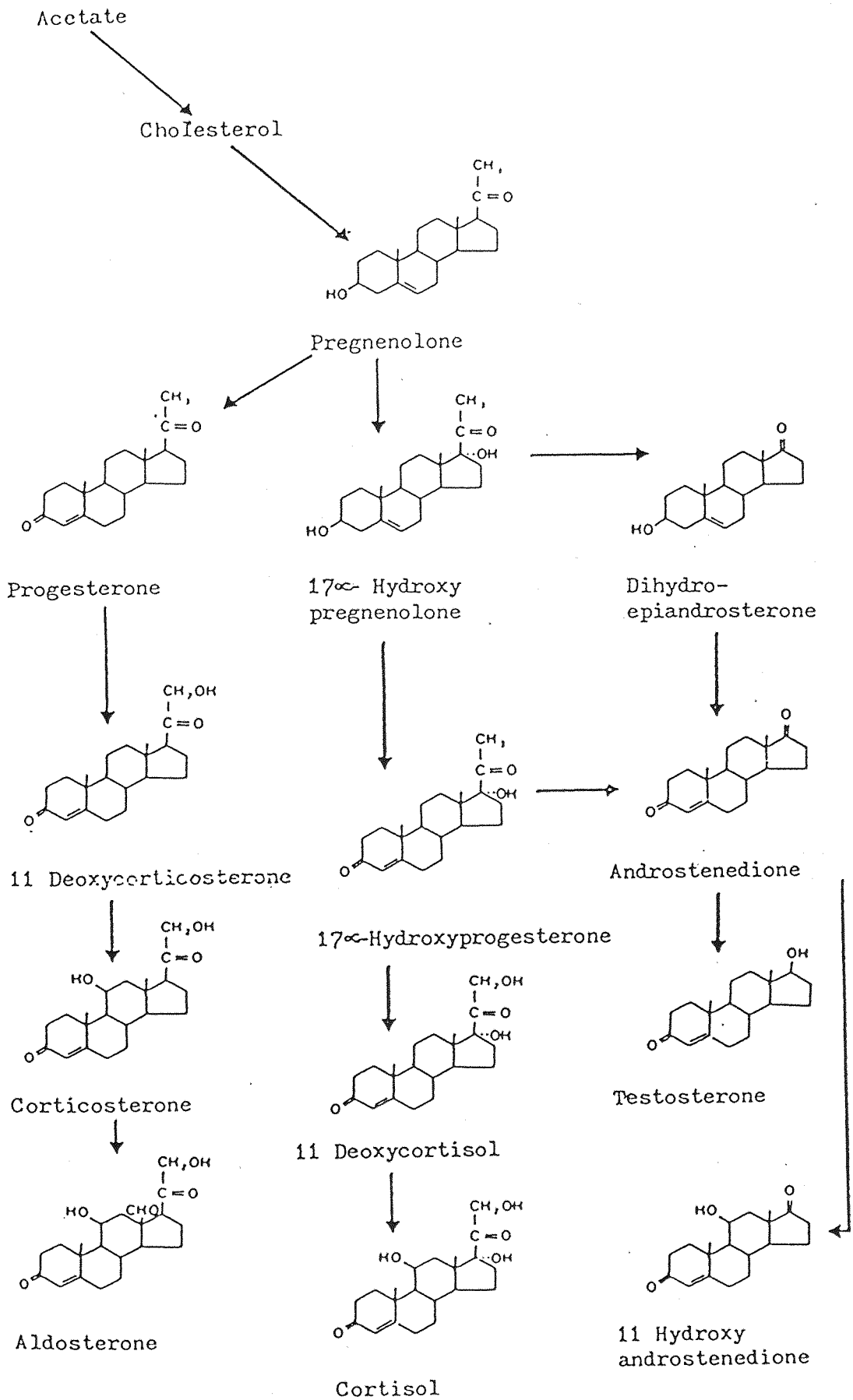


Fig. I.1. Steroid synthesis in the adrenal cortex.

expressed in the fetus. The major glucocorticoid secreted by the adult guinea pig, human and sheep is cortisol and by the rat is corticosterone.

The production of steroid depends upon the enzymes and also on the adrenocorticotrophic hormone (ACTH). The smallest anterior pituitary hormone, ACTH, is a polypeptide chain containing 39 amino acids (Mol. wt 4500 Daltons). The biologically active portion of the molecule resides in the 24 amino acids at the N-terminal end which is found to be common to many mammals (Imura, Sparks, Grodsky and Forsham, 1965) whereas the 24-39 amino acid region shows some species variation. ACTH secretion is stimulated by corticotrophin releasing factor (CRF) which is released from the hypothalamus. CRF reaches the anterior pituitary via the portal system of the hypothalamus-hypophysial tract.

The posterior pituitary peptide vasopressin (the antidiuretic hormone, ADH) has been suggested as a possible candidate for CRF (Harris, 1948). ADH is able to cause ACTH secretion both in vitro (Fleischer and Vale, 1968) and in vivo (Rivier, Vale and Guilleman, 1973). More recently Vale, Spiess, Rivier and Rivier, (1981) have isolated and characterised a 41 amino acid peptide which has CRF activity. Gillies, Linton and Lowry, (1982) reported that this peptide was more potent than ADH at releasing ACTH from pituitary columns. ACTH acts on the adrenal cell by binding to a receptor which is thought to be linked to the adenylate cyclase complex. Attachment of ACTH to the receptor activates the catalytic portion of the molecule. Adenylate cyclase catalyses the formation of cyclic AMP from ATP. This begins a chain reaction of enzyme steps which results in the synthesis and secretion of steroids from the cell.

The distribution of corticosteroids

Most of the cortisol in blood is found in the plasma and much of it is bound to proteins. Most species have specific corticosteroid binding globulin (CBG). This binding protects cortisol from degradation and renal filtration. CBG bound cortisol can dissociate and yield its steroid to tissues such as the liver (Paterson, 1973).

In sheep Paterson and Harrison (1968) have shown that the cortisol is probably present in the extracellular fluid of all tissues. More recently it has been shown that there are intracellular receptors specific to various steroid hormones. Corticosteroid receptors have been found in almost all tissues (Ballard, Baxter, Higgins, Rousseau and Tomkins, 1974).

The biological action of glucocorticoids

Glucocorticoids have widespread effects on most organs of the body to regulate metabolism of proteins, nucleic acids and fat as well as carbohydrates. There is a widely held view that glucocorticoids exert a "permissive" action on metabolism and enzyme function. The adrenals are involved in the body's response to stress i.e. exposure to noxious or potentially dangerous stimuli.

Glucocorticoids influence carbohydrate metabolism by promoting the conversion of protein to glucose. They thus maintain blood glucose by promoting gluconeogenesis, by inhibiting peripheral utilisation of glucose and increase glycogen deposition in the liver. The effects of glucocorticoids on most organs are catabolic. In muscle glucocorticoids inhibit protein and nucleic acid synthesis and enhance protein breakdown to provide amino acids for use by the liver. Glucocorticoids increase lipolysis and enhance the effects of other lipolytic stimuli such as catecholamines. They are required for the maintenance of normal vascular tone and hence a normal glomerular filtration rate.

Glucocorticoids have a number of actions on central nervous function. There is a considerable influence on the function of the sense organs. The discrimination of qualities of taste, smell and sound are impaired in adrenocortical insufficiency. For example "sweet" and "salty" can be discriminated only with sugar and salt solutions of much higher concentration than normal. Normal function can be restored by administration of glucocorticoids (Bruck 1983; Moran-Campbell, Dickinson, Slater, Edwards and Sikora, 1984; Lamb, Ingran, Johnston and Pitman, 1982).

The trophic control of adrenal development and function

The growth and functional activity of the fetal adrenal cortex is influenced by trophic factors. These factors are most likely to be of hypothalamic-pituitary origin. Many experiments have shown that removal of the pituitary in fetal rats, mice or rabbits by fetal decapitation or by destruction (Wells, 1947; Jost, 1966; Eguchi, 1961) stopped growth of the fetal adrenal glands. This change can be reversed by giving ACTH. The real significance of the hypophysial action on adrenal growth is given by comparative study of the growth of the rat adrenals in the presence and absence of the pituitary (Jost, 1966). In control animals the adrenal grows steadily between day 16.5 and 20.5 whereas in fetuses decapitated on day 16.5 the adrenals have atrophied by day 21.5. A similar type of pituitary control of adrenal growth was observed in rabbit fetuses (Jost, 1966). In ancephalic human fetuses premature regression of the fetal zone of the adrenal cortex in utero is seen (Benirceshke, 1956). Infusion of ACTH into pregnant monkeys increased the level of maternal cortisol and had no effect on fetal plasma cortisol while injection of ACTH directly into the fetus increased fetal adrenal secretion (Kittinger, Beamer, Hagemens, Hill, Bangham and Ochsmer, 1972). In humans the adrenal of the anencephalic fetus was unable to respond to an increment in plasma ACTH presumably because of functional atrophy (Allen, Greer, McGilvra, Castro and Fisher, 1974). In sheep Nathanielsz, Jack, Krane, Thomas, Ratter and Rees (1977) have demonstrated similar independance of maternal and fetal ACTH.

The functional relationship between the fetal pituitary and adrenal cortex is substantiated by feedback effects observed in the fetus. In rat fetuses an excess of corticosteroids in the fetus produced by giving hormones to the mother (Davis and Plotz, 1954) or by injecting hormones directly into the fetus (Jost, 1953) decrease the size of the fetal adrenal. This can be prevented by ACTH injection directly into the fetus. The excess of corticoids decrease the ACTH content of the fetal pituitary and adrenalectomizing the pregnant female rat results in adrenal hypertrophy of the young rat at term (Jost, 1975).

Nathanielsz, Comline, Silver and Paisey, (1972) suggested that certain observations on twin pregnancies in sheep support the existence of feedback loops in the fetal adrenal axis. When delivery was induced by the injection of cortisol into one twin, after a delay plasma cortisol concentration rose in the other twin. Evidence for the hypothalamic control of cortisol stimulating activity of the fetal pituitary. When the hypothalamus of the fetal rat was removed with the whole brain leaving the pituitary in situe on day 19.5 the adrenal of the encephalectomised fetuses was smaller on day 21.5 as in hypophysectomised fetuses (Jost 1975).

The regulation of cortisol concentration in the fetus

By term the fetal plasma cortisol concentration increases significantly in a number of species. In the human fetus by 22-24 weeks of gestation the adrenal can synthesise cortisol from progesterone derived from the placenta or from acetate. Cortisol biosynthesis independent of placental progesterone is necessary for extra-uterine life (Nathanielsz, 1976). Recent information suggests that regulation of the fetal pituitary-adrenal axis is also dependent on placental function. Clearly cortisol production is carefully regulated by the fetus and placenta. Plasma concentrations of cortisol depend on four variables (Barr, Lugg and Nicholas, 1980, Anderson and Winter, 1985) :-

1. Pituitary ACTH secretion.

2. Inhibition of 3 β HSD (Hydroxysteroid oxidoreductase) by placental and adrenal steroids.

3. Rapid placental clearance of cortisol by conversion to cortisone.

4. Placental transport of fetal and maternal cortisol

Maternal cortisol readily crosses the placenta and is converted by placental 11β hydroxysteroid oxidoreductase (11β HSD) to inactive

cortisone (Murphy, 1981). Uteroplacental cortisol metabolism changes from oxidation at mid gestation to reduction in late gestation. In human fetuses at mid term conversion in vivo of cortisol to cortisone (oxidation) was shown in several tissues including lung, liver and kidney (Pasqualini, Nguyen, Uhrich, Wiquist and Diczfalusy, 1970). The same group showed that in guinea pigs similar conversion of cortisol to cortisone occurred but at term in the tissues cortisone was converted to cortisol (reduction). In the fetal guinea pig the increase in cortisol concentration could be due to an increased development of 11β hydroxysteroid oxidoreductase which transforms cortisone to cortisol during the last days of pregnancy (Dalle, Pradier and Delost, 1983).

The species shown so far to have high placental and fetal oxidative 11β HSD activity include man, guinea pig, rat and mouse - all species having a haemomonochorial type of placentation. This fetal 11β HSD activity occurs in a number of tissues including liver, muscle, placenta, kidneys, lungs, testis, ovary, skin, adrenal and connective tissue. Circulating cortisone has not been reported in fetal sheep which have a syndesmochorial placentation (Murphy, 1981). Murphy (1979) suggests that conversion of cortisol to cortisone in the fetus protects the fetus against the growth inhibiting effects of cortisol while production of cortisol from cortisone in the membranes and uterine wall contributes to the mechanisms which prevent the fetus being rejected by the maternal immune system.

Fetal cortisol - role in fetal development

In experimental animals fetal glucocorticoids have been shown to stimulate enzymes responsible for gluconeogenesis and hepatic glycogen accumulation, induce enzyme activity of intestinal epithelium and maturation of pancreatic beta cells, promote development of type II alveolar cells and synthesis of pulmonary surfactant, increase phenylethanolamine N methyl transferase activity in the adrenal medulla, and enhance triiodothyronine synthesis from thyroxine. Thus they play an important role in the maintenance of fetal homeostasis

and the preparation for birth in several species (Liggins, 1976; Mitchell, Seron, Hess and Jaffe, 1981), and contribute to the initiation of lactation in the rabbit and mouse (Gala and Westphal, 1967).

Fetal cortisol and parturition

For a long time the fetus was considered to play no role in the termination of pregnancy and in delivery on the basis of experiments performed on rats, mice and monkeys involving surgical removal of the fetus (Kirsch, 1938; Selye, Collip and Thompson, 1935; Newton, 1935; Van Wagenen and Newton, 1943). The placentae left in situe are often said to have been reported as being delivered on time however Nathanielsz (1976) points out that this is not substantiated by a careful analysis of the original reports. An active part is played by the fetal endocrine glands, especially by the fetal pituitary-adrenal axis. In sheep fetuses and in other ruminants fetal plasma cortisol concentration rises during late gestation before the onset of labour. This rise in cortisol production provides an essential signal for parturition in these species (Bassett and Thorburn, 1969). In the human the fetal adrenal may be important in the precise timing of parturition (Anderson and Winter, 1985). In at least one species, the sheep, fetal cortisol can trigger the mechanism leading to the onset of parturition (Liggins, Kennedy and Holm, 1967). Fetal hypophysectomy causes adrenal hypoplasia and prolongs pregnancy in sheep (Liggins et al, 1967) goats (Rawlings and Ward, 1978) and pigs (Lohse and First, 1981) and infusion of ACTH or glucocorticoids into the fetus will increase fetal cortisol concentrations and induce parturition in the sheep (Liggins, 1968; Liggins, Fairclough, Grieves, Kendall and Knox, 1973), goat (Currie and Thorburn, 1977) cow (Welch, Forst and Bergman, 1973) and pig (Lohse and First, 1981)

It has been shown that administration of synthetic glucocorticoids such as dexamethasone to pregnant animals will result in the appearance of the drug in the fetal circulation (Anderson, Stock and Rankin, 1979; Funkhauser, Peevy, Muckride and Hughes, 1978).

The placental permeability to dexamethasone is greater than to cortisol in the maternal to fetal direction (Comline, Hall, Lavelle, Nathanielsz and Silver, 1974). Maternal administration of dexamethasone does not result in premature delivery in guinea pigs (Illingworth, Challis, Ackland, Burton, Heap and Perry, 1974; Donovan and Peddie, 1974) monkeys (Novy and Walsh, 1983), horses (Adam and Wagner, 1970) and women (Warren and Cheatum, 1967). Cortisol does cause premature parturition in the rabbit when given to the mother (Nathanielsz, Abel and Smith, 1973a). Administration of dexamethasone to pregnant cows can induce premature delivery five to six days later (Comline et al, 1974). In sheep dexamethasone will induce parturition when infused into the mother for 2 to 3 days but only very large doses are effective (Adams and Wagner, 1970)

The Thyroid Gland

The normal adult thyroid gland is composed of two lobes joined by a midline isthmus. The gland is located in the lower part of the neck anterior to the trachea between the sternocleidomastoid muscles. It receives an extensive arterial blood supply from the superior and inferior thyroid arteries on each side. In histological sections the thyroid gland exhibits many large cavities called follicles. The follicle wall is formed by a layer of cuboidal epithelial cells. The shape of follicular cells varies among species. The follicles are filled with a viscous protein solution called colloid which contains the hormones thyroxine (T4) and triiodothyronine (T3). In the spaces between the follicles are so-called parafollicular cells (C Cells) which produce calcitonin or thyrocalcitonin. Calcitonin is concerned with the regulation of calcium homeostasis and will not be considered further here. The inter-follicular spaces also contain a dense network of capillaries by which the components for hormone formation are supplied and the completed hormones are removed. The blood vessels between the follicles have a rich sympathetic innervation. The thyroid receives a parasympathetic nerve supply but its distribution is not well documented.

The follicle is the smallest functional unit of the thyroid. The iodine containing thyroid hormones are synthesised from the large glycoprotein thyroglobulin. Thyroglobulin in the normal gland is present as a soluble protein stored in the follicular lumen. It has a high content of iodinated amino acids. The iodotyrosines, which form the most abundant iodinated amino acid component of thyroglobulin, are themselves without physiological activity. They are precursors of the biologically active hormones T4 and T3. T4, the major secretory product of the thyroid gland, consists of two phenyl rings linked via an ether bridge with an alanine side chain on the inner ring. It contains four iodine atoms attached at carbons 3 and 5 of the inner ring and 3' and 5' of the outer ring.

The hormone 3,3',5-triiodothyronine (T3) is derived from T4 by 5'

deiodination of the outer phenolic ring of the molecule, a process known as β -deiodination. Deiodination in the 5 position of the inner tyrosyl ring, α -deiodination, results in the production of 3,3',5'-triiodothyronine, reverse T3 (rT3) (Fig. I:2)

Hormone synthesis occurs in the thyroid follicular cells, which exhibit marked functional polarity from their basal to their apical sides, and in the colloid. The biosynthetic process shown in Fig I:3 has been divided into a number of steps. The first step is active transport of iodide into the follicular cell. This process occurs at the basal surface and requires ATP. Iodide is actively concentrated by the thyroid gland. When the plasma iodide concentration is low the ratio of thyroid gland to plasma iodide concentration may exceed 100. Iodide is also concentrated,to small extent,by salivary gland, gastric mucosa, small intestines, skin, mammary tissues and by the placenta. On the apical surface of the follicular cells iodide is rapidly oxidised to iodine and incorporated into tyrosine residues in the thyroglobulin molecules. This results in the formation of monoiodotyrosine (MIT) or diiodotyrosine (DIT) within the protein. There is subsequent coupling of these iodinated tyrosines in the thyroglobulin molecule to yield T4 and T3 (Gross and Pitt-Rivers, 1952). The reactions are catalysed by thyroid peroxidase and involve the formation of hydrogen peroxide.

Thyroglobulin, a large glycoprotein, is mainly synthesised in the rough endoplasmic reticulum of the follicular cells (Tice and Wollman, 1974). Normally iodinated thyroglobulin contains about 10 to 50 atoms of iodine which are distributed between MIT and DIT and the two hormones T4 and T3 (Edelhoch and Robbins, 1978). Thyroglobulin is stored as a colloid in the lumen of the thyroid follicles. Formation of active thyroid hormones requires reabsorption of colloid by endocystosis, fusion of the vesicles with lysosomes and degradation of the thyroglobulin by lysosomal protease. T4 and T3 are released and diffuse into the blood. The iodine in the uncoupled MIT and DIT is reclaimed for hormone biosynthesis by the thyroid deiodinase enzymes.

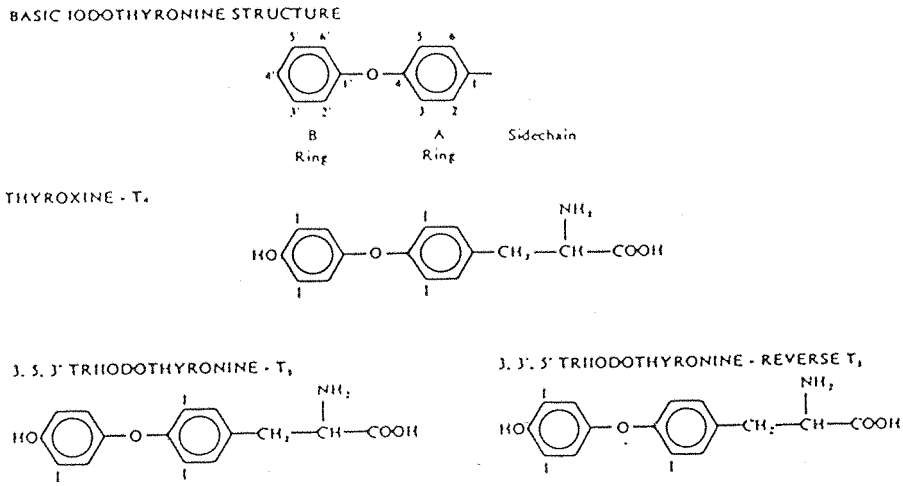


Fig:I-2 THE CHEMICAL STRUCTURE OF THE THYROID HORMONES.

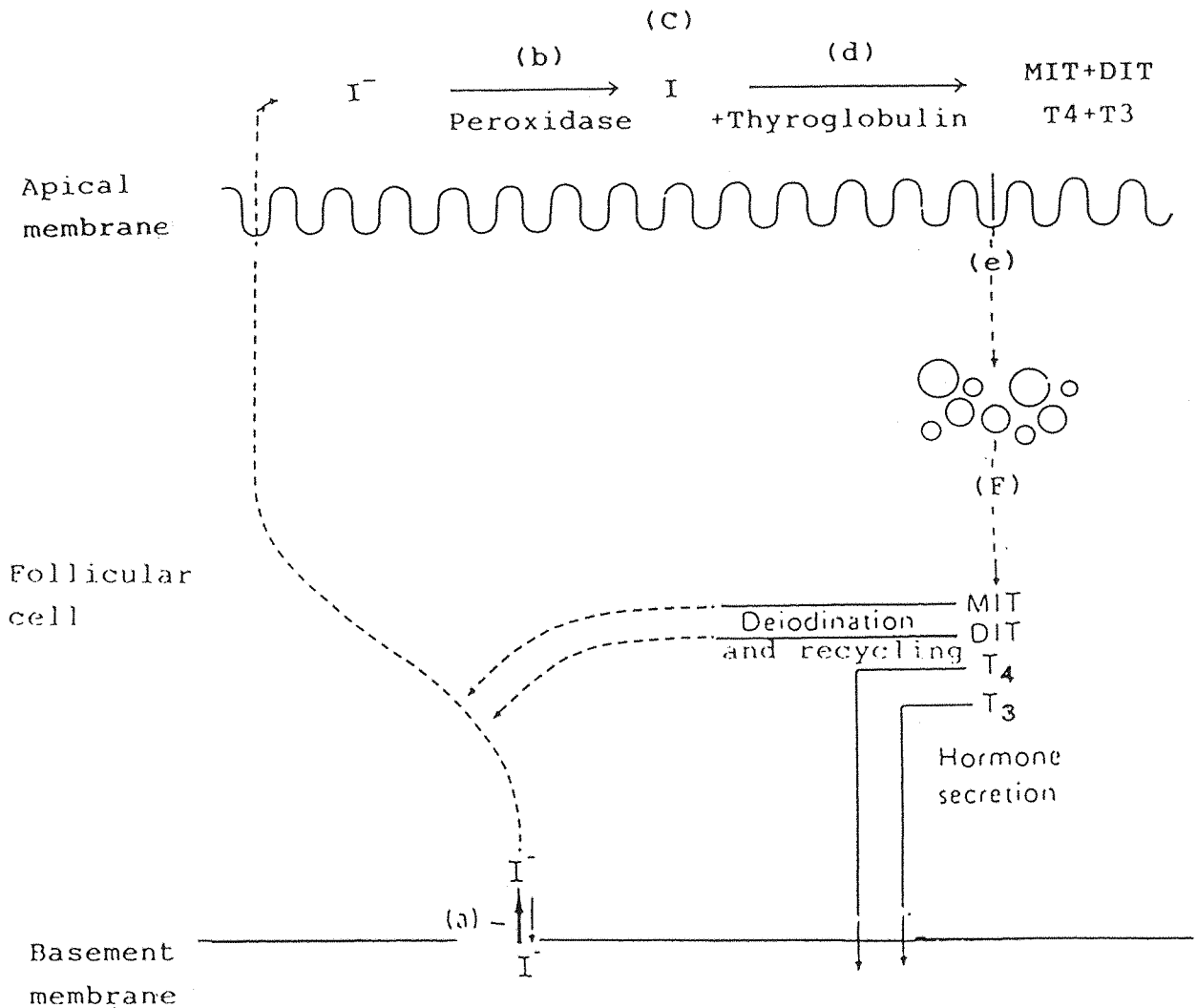


FIG: I-3 THYROID HORMONE BIOSYNTHESIS AND RELEASE

a- Active transport b- Iodide oxidation
c- Activated iodine d- Iodination and coupling
e- Colloid resorption f- Thyroglobulin proteolysis

The anterior pituitary gland secretes a thyroid stimulating hormone (TSH) which stimulates all thyroid function. Central nervous system (CNS) control is exercised via the hypothalamic neurohormone thyrotrophin releasing hormone (TRH) which binds to a specific cell surface receptor on the pituitary thyrotrophs and stimulates TSH synthesis and secretion. The glycoprotein TSH consists of α and β sub units. The α sub-unit is related to that in LH and FSH.

TSH binds to specific high affinity cell surface receptors on thyroid cells and activates adenylate cyclase so increasing cellular concentrations of cyclic adenosine 3',5' monophosphate (cAMP). TSH increases the entire pathway of hormone biosynthesis including an increase in iodide trapping from the plasma, increased synthesis and iodination of thyroglobulin and an increased rate of degradation of thyroglobulin. The resulting increase in the secretion of T4 and T3 is accompanied by an increased blood flow through the gland. The thyroid hormones exert a negative feedback control at the level of the pituitary thyrotroph (Vale, Burgus and Guillemam, 1968). Variations in the circulating concentration of thyroid hormones are the principal regulator of TSH secretion through this feedback loop. Reichlin, Martin, Mitnick, Bosh, Grimm, Bollinger, Gordon and Malacura (1972) have suggested that T4 may have a positive feedback effect upon hypothalamic TRH production. It is generally considered that TSH secretion is a balance between the positive effects of TRH from the hypothalamus determining the set point of the system and negative feedback of the plasma T4 concentration on the pituitary cells. The mechanism of action of the β deiodinase enzymes in the pituitary is such that most of the available T4 is converted to T3. Balfour (1969) demonstrated that inhibition of TSH secretion was more closely related to pituitary T3 content than pituitary T4 content. Friedman, Lang and Burk (1977) have reported that thyroid hormones directly inhibited the thyroid adenyl cyclase and the thyroid response to cAMP.

The peripheral conversion of T4 to T3 is another site for regulation of the thyroid axis. It is important because it directly

regulates the amount of active hormone present in the tissue. Increased sympathetic activity is known to enhance T4 to T3 conversion. Wiersinga, Modderman and Touber (1980) showed that α and β agonists increased the in vitro T4 to T3 conversion. Starvation (Vagenakis, Burger, Portnay, Rudolph, O'Brian, Azizi, Arky, Nicod, Ingbar and Braverman, 1975) and glucocorticoid treatment (Chopra, William, Orgazzi and Solomon, 1975a) depress T4 to T3 conversion. This may have important physiological consequences during starvation and glucocorticoid therapy when plasma T3 concentrations are reduced and plasma rT3 concentrations are increased (Chopra et al, 1975a).

In most species total plasma T4 concentrations are usually approximately 100 times as great as the total plasma T3 concentrations. In humans the total plasma T4 concentration is about 80 ng/ml while total plasma T3 concentration is about 1.5 ng/ml (Singer and Nicoloff, 1972). In adult sheep Thomas and Nathanielsz (1983) reported that plasma T4 concentrations were 60 ng/ml and T3 0.6 ng/ml. In adult guinea pigs the plasma T4 concentration is 22 ng/ml and T3 is 0.5 ng/ml (Leppaluoto, Huttunen and Hirvonen, 1981). In the non-pregnant ewe the plasma T4 concentration is 126.61 $\pm$ 1.14 ng/ml, in the cow 67.9 $\pm$ 4.6, in goat 120.8 $\pm$ 5.7, in horse 22.2 $\pm$ 3, in rat 62.0 $\pm$ 1.0 and in guinea pig 23.7 $\pm$ 0.21 ng/ml (Etta, 1973).

Upon secretion into the circulation T4 and T3 are tightly bound to serum proteins so that only a small fraction of the total amount circulating is in the free form and able to enter cells and exert metabolic actions. Protein bound hormone serves as a protected reservoir which is not subject to renal clearance and buffers the plasma free hormone concentration. T4 is bound to three proteins thyroxine binding globulin (TBG), thyroxine binding pre-albumin (TBPA) and albumin all of which are synthesised in the liver. In man TBG, a glycoprotein with a molecular weight of 55,000 Daltons, has the highest affinity for thyroid hormones. It binds approximately 75 % of T4 and 50 % of T3 in the circulation. TBPA has a lower affinity for T4

binding but has a much greater capacity. Albumin has a very low affinity but a very great capacity. Protein binding, principally to TBG results in a long half-life of plasma thyroid hormones. T4 has a half-life of about 7 days in man whilst T3, which is bound less strongly, has a half-life of 1 to 3 days. In the rat T4 has a half-life of 14 hours while that for T3 is about 7 hours (Schwartz, Surks and Oppenheimer, 1971). In guinea pig plasma T4 was found to bind only to plasma albumin (Tanate, Ishil and Thomaki, 1969; Etta, 1973). In neonatal guinea pigs Pals, Reinke and Shaw (1973) measured a T4 half-life of about 1.23 days (30 hours). In sheep the mean T4 half-life in non-pregnant ewes is 37 hours (Dussault, Hobel and Fisher, 1971).

During pregnancy TBG concentrations increase because of oestrogen stimulated TBG production. When TBG levels increase the initial effect will be a shift in hormone from the free to the bound state. The total concentration will increase first because of a decreased metabolic clearance rate (MCR) and secondly because of increased thyroid hormone production as the hypothalamic-pituitary-thyroid system responds to the fall in free hormone concentration.

The metabolism of thyroid hormones, which occurs predominantly in the liver, is catalysed by 5'-deiodinase, a microsomal enzyme which requires reduced sulphydryl groups from glutathione and other compounds for activity. Also T4 undergoes deiodination in the inner ring to yield the inactive metabolite reverse T3 (rT3). Inactivation of hormone occurs by progressive deiodination of the iodothyronine molecule. The breakdown products are then excreted in either the bile or urine with the former route predominating in man. Both T4 and T3 are excreted as glucuronide conjugates in the bile. Some of the biliary T4 is deconjugated in the intestine and reabsorbed but the extent to which this occurs depends to some extent on the intestinal flora and the bulk of the intestinal contents (Van Middlesworth, 1957).

Biological Effects of Thyroid Hormones

The hormones T4 and T3 have basically the same actions but T3 is about five times as effective as T4. T4 can be converted to T3 in the peripheral tissues by deiodination. For this reason it has been suggested that T4 might be regarded as a prohormone for the "actual" hormone T3 (Strelin and Lazaris, 1977). Both T4 and T3 effect various metabolic processes. In the young they promote growth and physical development particularly development of the nervous system. One of the most important actions of the hormones T4 and T3 is their effect on energy metabolism, their "Calorigenic Action". These hormones stimulate the basal rate of metabolism, oxygen consumption and heat production. T3 induced increase in oxygen consumption in the liver is accompanied by increased activity of the sodium/potassium ATPase. According to the studies of Edelman and Ismail-Beigi (1974) thyroxine activates the sodium pump which, in maintaining the extracellular and intracellular electrolyte concentrations normally accounts for 30 to 40 percent of the basal metabolic rate. This calorigenic action is important in cold exposure and thyroid hormones are essential for small animals to tolerate prolonged exposure to cold. Thyroid hormone secretion is increased during cold exposure and the rate of conversion of T4 to more metabolically active T3 is increased. However when adult guinea pigs were exposed to cold (-20 °C) serum T3 levels remained similar to that of the control animals but serum T4 was significantly higher ($P < 0.01$). In cold exposed animals the activation of the thyroid gland was preceded by an increase in oxygen consumption and a decrease in rectal temperature (Lappaluoto et al, 1981). It may be that this severe cold exposure constituted a stress and thus inhibited β deiodination.

Thyroid hormones cause increased incorporation of amino acids into protein (Buchanan, Primack and Tapley, 1971) and modifications in the turnover of mitochondrial DNA and protein. T4 induces cytoplasmic proteins which act at the mitochondrial level (Herd, Kaplay and Sanadi, 1974) and mitochondrial protein, molecular weight 54,000 Daltons can be induced by T3 (Baudry, Clot, Bouhnik, Michel and

Michel, 1975). These observations suggested a possible physiological action of thyroid hormones at the mitochondrial level.

Thyroid hormones have actions on lipids, proteins and carbohydrate metabolism. They stimulate the synthesis, mobilisation and particularly the degradation of lipid. They decrease lipid stores and the plasma lipid concentration by enhancing the actions of lipolytic agents (Bressler and Wittels, 1966). Also thyroid hormones increase the turnover of low density lipoproteins (LDL) which bind plasma cholesterol (Walton, Scott, Dykes and Davies, 1965). They modulate carbohydrate metabolism by affecting the actions of other hormones such as catecholamines. Administration of small amounts of thyroid hormone cause increased glycogen synthesis whilst larger amounts induce glycogenolysis. Also they increase absorption of glucose which exceeds deposition, insulin release is delayed and its turnover may be enhanced (Elgee and Williams, 1955). In guinea pigs the plasma free fatty acid (FFA) concentration and phospholipid and cholesterol concentrations were increased significantly after T3 injection but glucose-6-phosphate phosphohydrolase activity was not affected after thyroxine administration (Lee, Liu and Hsu, 1970).

Thyroid hormones are required for full maintenance of the function of the nervous system and in their absence axonal and dendritic development and myelination of the nervous system are defective. Although a lack of these hormones is known to cause an impairment of brain function and decreased responsiveness of the brain the exact mechanism of action is unclear. Surgical thyroidectomy has been performed on fetal sheep during the last third of gestation. The thyroidectomised lambs at birth were characterised by retarded brain development which was established by the reduced amounts of DNA, RNA and protein which were measured (Potter, McIntosh, Mano, Baghurs, Chavadej, Hua, Gragg and Hetzel, 1986). Neonatally throidectomised rats show a decrease in axonal and dendritic development, a decrease in total brain protein synthesis and a decrease in average cell mass (Thomas and Nathanielsz, 1983). Administration of T4 to neonatally thyroidectomised rats before the tenth postnatal day is able to restore almost normal levels of enzyme activity in the cerebral cortex (Gomez, 1971).

Antithyroid Compounds

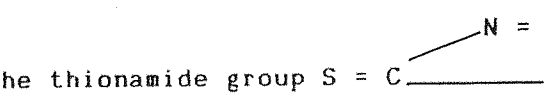
The term antithyroid drug refers to a specific group of compounds that block the synthesis of thyroid hormones. In general it is synonymous with goitrogen. Antithyroid drugs lower the level of thyroid hormone in the circulation and thus produce goitre by increasing the secretion of TSH. TSH can stimulate thyroid growth directly without stimulating thyroid hormone synthesis (Yamada, Kajihara, Takemura and Onaya, 1975). The mechanism of action of goitrogens are :-

1. Compounds which interfere with synthesis of thyroid hormones

2. Compounds which inhibit accumulation of iodide by the thyroid gland

3. Compounds which augment faecal loss of thyroid hormone

1. Compounds which inhibit thyroid hormone synthesis such as thionamides

All thionamides contain the thionamide group  and include derivatives of thiouracil and methimazole. The antithyroid drugs such as PTU (propylthiouracil) have a specific inhibitory effect on the iodotyrosine coupling reaction. It was found that low concentrations of PTU greatly inhibited thyroxine formation without decreasing DIT or MIT formation. Taurog (1970) has shown that thionamides are inhibitors of peroxidase catalysed iodination. In vivo studies indicated that synthesis of thyroid hormones is inhibited by goitrogens in the following order as dosage increases.

a. coupling of iodotyrosines to form T4 and T3

b. formation of diiodotyrosine

c. formation of monoiodotyrosine (Iino, Yamada and Greer, 1961; Pasternak, Socolow and Ingbar, 1969). Wolff, Chaikoff, Coldberg and

Meier (1949) have shown that large doses of iodide can block organic binding of iodine in normal rat thyroid glands.

2. Compounds which inhibit accumulation of iodide by the thyroid gland.

Wolff, Chaikoff, Taurog and Rubin (1946) and Vanderlaan and Vanderlaan (1947) established that thiocyanate inhibits the ability of the thyroid gland to concentrate inorganic iodide. Perchlorate, fluoborate and nitrate can also block organic binding of iodine by the thyroid (Greer, Stott and Milne, 1966). Treatment of hypertension with thiocyanate produced goitre and hypothyroidism in man. Subsequent studies demonstrated that this effect of thiocyanate could be prevented by administration of either thyroid hormone or iodide (Yamada et al, 1975).

3. Compounds which augment faecal loss of thyroid hormone.

Goitre and hypothyroidism have been reported in infants fed a soy-milk diet but both disappeared when the soy milk diet was discontinued (Van Wyk, Arnold, Wynn and Petter, 1959). Van Middlesworth (1957) has reported an increased faecal loss of thyroxine in soy fed rats. It seems that soy bean is goitrogenic because of an increased faecal loss of thyroid hormone thus increasing pituitary-thyroid activity to maintain normal blood thyroid hormone levels.

The Fetal Thyroid

The embryological differentiation of the gland in the mammal follows a similar series of steps in all species (Nathanielsz, 1976). The thyroid of the human embryo derives as a midline outpouching of the endoderm of the floor of the primitive buccal cavity. The endodermal thyroid develops as a flask like vesicle with a narrow neck which, at 24 days, still connects with the buccal cavity. By 30-40 days the thyroid becomes a solid mass and by 45-50 days the gland has descended to its definitive location in the anterior lower neck. The gland develops in three phases: The precolloid, beginning colloid and follicular growth phases. These occur at 47-72, 73-80 and beyond 80 days respectively. Thyroglobulin is present at 29 days but fetal TSH is not detectable until 70 days when the fetal thyroid gland is capable of concentratng iodide and synthesising thyroxine (Fisher, Dussault, Sack and Chopra, 1977). In the fetal rat thyroid development does not appear to be TSH dependent. The gland will develop histologically and store colloid as well as a small amount of hormone in the absence of the pituitary. The first colloid droplets are seen by day 17 and the uptake of iodide and the presence of follicles is seen by day 18 (Fisher et al, 1977). In the fetal sheep thyroglobulin production begins at about 50 days and iodothyronines first appear at about 70 days. The early growth and histological development of the gland does not depend on fetal TSH (Thomas and Nathanielsz, 1983). At 100 days the fetal sheep thyroid is relatively large (462 mg/kg body weight) but the rate of growth is less during the last third of gestation so that near term its relative size has decreased by about 50 % to 184 mg/kg body weight (Nathanielsz, Comline, Silver and Thomas, 1973b).

In fetal monkeys follicle formation and colloid synthesis were apparent by 50 days of gestation when ^{131}I uptake is just detectable. At this age the fetal body weight was 36.6 g and the ratio of thyroid weight to body weight was 0.317 (mg thyroid weight/g body weight). At 75 days colloid was present in significant amounts

and the total gland weight averaged 10 mg. Between 75 and 150 days the weight of the gland increased only ten fold such that by 150 days average body weight was 153 g and the thyroid weight to body weight ratio was 0.245 mg/g (Pickering and Kontaxis, 1961; Pickering, 1968).

In the pig at a fetal age of 50 days most of the gland already showed small follicles filled with colloid. At 75 days the resorbtion vacuoles in the colloid became more apparent. These were first seen in some animals at 60 days. At 110 days the gland had quite large follicles. The fetal pig pituitary can produce TSH from 70 to 76 days. Thyroid gland weight increased between 50 and 110 days from 1.3 ± 0.5 mg to 130 ± 35.0 mg (Mean ± S.D.) (Fentener-Van Vlissingen, Colenbrander and Wensing, 1983).

Placental Permeability to Hormones

Placental permeability to thyroid hormones has attracted the attention of many authors. Numerous studies have been carried out in a number of species. The ability of thyroid hormone treatment of the mother to prevent or to eliminate the production of fetal goitres by goitrogens administered to the mother has been used as a method of determining the placental permeability to the hormones. Mitskevich (1962) used the action of well known antithyroid drugs and thyreoidin. His study was carried out in rabbits on days 20, 24, 27 and 30 of gestation and on pregnant guinea pigs. The rabbits were fed methylthiouracil and given thyroid hormone replacement from the onset of pregnancy. Thyroids of 24 day old fetuses showed a response to methylthiouracil despite the maternal administration of thyroid hormone. At day 27 however thyroid hormone was able to pass the placenta from mother to fetus and prevent fetal goitre. Guinea pigs were shown to possess a placenta which permitted the passage of thyroid hormone at about 35 days of pregnancy i.e. at a stage close to the onset of functional activity of the fetal thyroid.

Peterson and Young (1952) have found a similar result in guinea pig fetuses in mothers fed PTU with or without thyroid hormone until

term. They concluded that T4 crossed the placenta to inhibit fetal TSH secretion. Postel (1957) in his study of PTU treated pregnant guinea pigs found little or no radioactivity in the blood of fetuses in pregnant guinea pigs which had been injected daily from 3-7 weeks before normal term with 0, 8, 16 or 32 µg of T3 and which recieved an injection of radioactively labelled T3 24 hours before sampling. Administration of large doses of T4 (200 µg/day) to pregnant rats will prevent the development of PTU induced goitre in the fetus (Hoskins, VanArsdel and Williams, 1958).

Data from human studies have been reported by Grumbach and Werner (1956) who produced evidence that at term the human placenta is only slightly permeable to labelled thyroxine. Azukizawa, Murata, Ikenoue, Martin and Hershman (1976) have reported that TRH can cross the monkey placenta in either the maternal to fetal or fetal to maternal direction. The fetal thyroid during the latter part of gestation can respond to TSH and release both T4 and T3 and the fetal response to TSH was significantly greater than that of the maternal thyroid.

It appears that no components of the thyroid system are able to cross the placenta in significant amounts in the sheep (Thomas, Jack, Manns and Nathanielsz, 1975). The administration of 200 µg of TRH to the mother produced a prompt increase in maternal TSH but no change in any hormones of the fetal thyroid system. Similarly the administration of 50 µg of TRH to the fetus was without effect in the mother. These observations indicated that their was no transfer of endogenous hormones or of the administered TRH. A rapid disappearance of plasma T4 and T3 and increase in plasma TSH in thyroidectomised sheep fetuses has been observed by Thorburn and Hopkins (1973).

These data show that placental permeability depends upon the type of placenta and the stage of the pregnancy. It seems that haemoendothelial and haemochorial types of placentae are most easily penetrable.

Fetal and Maternal Hormone Concentrations During Gestation

In rats no thyroxine can be detected in 16-17 day old fetuses. It becomes measurable at day 18 and by day 20 has reached 6.56 ± 0.13 nmol/l. In the mother plasma T4 concentration increased progressively from 3.48 ± 0.13 nmol/l to 11.33 ±0.90 nmol/l at birth (Wrutniak and Cabello, 1983). The thyroxine concentration was significantly lower in the fetuses than the mothers (Pic and Bonguin, 1985). In fetal pigs plasma T4 levels increased from about 0.06 µg/100 ml at a fetal age of 42 days to 4.19 ± 0.88 µg/100 ml at 110 days. The highest plasma T4 concentration was measured between 64 and 90 days. In pregnant sows plasma T4 levels at 42-72 days of gestation were between 1.97 and 3.57 µg/100 ml (Fentener-Van Vlissingen et al, 1983). Fetal plasma TSH concentration averaged 0.6 ± 0.2 ng/ml and showed no remarkable changes either before birth or in the early postnatal period. Plasma T3 concentration between 90 and 109 days of gestation in fetal pigs averaged 98.2 ± 20.1 ng/100 ml. In the late fetal period the T3 concentration rose to 159 ± 12.9 ng/100 ml ($P < 0.01$). Colenbrander, Macdonald, Wong and Parvizi (1980) reported that plasma T4 concentration remained relatively constant during the late fetal period and averaged 7.9 ± 0.7 µg/100 ml.

In the human the maternal serum T4 concentration is elevated during pregnancy. The mean serum T4 concentration between 11 and 18 weeks (12.9 ± 1.1 µg/100 ml) is similar to that between 22 and 34 weeks (12.2 ± 0.51 µg/100 ml). The mean maternal serum concentrations of TSH between 11 and 18 weeks (4.2 ± 0.68 µU/ml) is also similar to that between 22 and 34 weeks (3.8 ± 0.38 µU/ml). The concentrations of free T4 in maternal serum at 11 to 18 weeks (2.97 ± 0.27 ng/100 ml) and at 22 to 34 weeks (2.82 ± 0.12 ng/100 ml) are similar. Fetal serum T4 concentrations are low in the 11 to 18 week fetuses (1.9 to 3.9 µg/100 ml). The mean levels increase progressively to term (7.2 ± 0.61 µg/100 ml at 22 to 34 weeks). A similar progressive increase in serum free T4 concentration is observed. The proportion of T4 which is free is high in 11 to 18 week fetuses (0.054 % to 0.095 %, mean 0.077 %). The mean concentration of free T4 (1.85 ± 1.7 ng/100 ml) is significantly less ($P < 0.01$) than

that in the 22 to 34 week fetuses (2.49 ± 0.17 ng/100 ml). Serum TSH concentration in the 11 to 18 week old fetuses vary from 0 to 4 µU/ml and between 22 to 34 weeks they vary from 2.4 to 20 µU/ml (Fisher, Hobel, Garza and Pierce, 1970). At term in cord serum and maternal serum the mean T4 levels were 9.4 ± 0.5 µg/100 ml and 10.8 ± 0.4 µg/100 ml respectively. The mean T3 concentrations were 150 ± 8 ng/100 ml in maternal sera and 30 ± 3 ng/100 ml in cord sera. The mean rT3 concentration in cord sera was 315 ± 16 ng/100 ml and in pregnant women at term rT3 was 79 ± 5 ng/100 ml (Burman, Read, Dimond, Strum, Wright, Patow, Earll and Wartofsky, 1976).

In non-pregnant female monkeys the levels of plasma TSH, T4 and T3 are 0.95 ± 0.21 µU/ml, 6.0 ± 1.3 µg/100 ml and 112 ± 13 ng/100 ml respectively. The plasma concentrations of TSH, T4 and T3 of the pregnant monkeys did not differ from those of non-pregnant animals the mean plasma concentrations between 115 and 145 days of gestation being 1.1 ± 0.2 µU/ml, 5.2 ± 0.5 µg/100 ml and 142 ± 9 ng/100 ml respectively. In the fetuses over the same range of gestational age the mean plasma TSH, T4 and T3 concentrations were 4.0 ± 1.1 µU/ml, 5.7 ± 0.7 ug/100 ml and 54 ± 11 ng/100 ml (Azukizawa et al, 1976).

In the fetal sheep plasma T4 first becomes detectable at about 65 days of gestation after which the plasma concentration gradually rises to levels which vary between 45 to 100 ng/ml in different fetuses. After day 135 of gestation there is a fall in T4 concentration in some fetuses during the 7 to 10 days before delivery. Free T4 is constant at 30 to 100 pg/ml. Both total and free plasma thyroxine concentrations exceed those of the mother. Fetal plasma T3 concentrations are low and are often unmeasurable throughout most of gestation only beginning to increase slightly during the last eight to ten days of gestation while maternal T3 concentrations are of the order of 2.0 ng/ml. Fetal plasma rT3 concentrations showed similar changes to those of T4. There was no significant change in the ratio of fetal plasma T4 to fetal plasma rT3 over the last third of gestation (Mathur, Brown, Krane, Thomas and Nathanielsz, 1980). In the fetal calf a similar situation is to be found. Plasma T4 concentrations are high and exceed those of the mother while fetal

plasma T3 concentrations are low or unmeasurable (Thomas and Nathanielsz, 1982).

In the fetal horse Irvine and Evans (1975) reported that the total T4 and free T4 levels in cord blood (38.4 ng/ml and 13.4 ng/100 ml respectively) were considerably higher than in jugular blood taken from the foal one to ten hours after birth (total T4 28.9 ng/ml), whereas total T3 and free T3 in cord blood (529 ng/100 ml and 1.42 ng/100 ml) were markedly lower than in jugular blood taken one to ten hours later (991 ng/100 ml for T3).

There are few data concerning thyroid function in the fetal guinea pig. Jones, Lafeber and Roebuck (1985) in a study principally concerned with experimental growth retardation reported that fetal guinea pig T4 concentrations were much higher than those of T3 or rT3 and that they increased progressively from 40 days to reach a peak at 50-55 days and then gradually declined towards term. The T3 and rT3 concentrations showed similar changes to those of T4. Recently Castro, Alex, Young, Braverman and Emerson (1986) have reported a study of thyroid hormone concentrations in the adult and fetal guinea pig. Total serum T4 levels in adult males, non-pregnant females and pregnant females did not differ significantly (range 2.5 $\pm$ 0.3 to 3.2 $\pm$ 0.8 µg/100 ml; Mean $\pm$ S.D.). Similarly there were no significant differences in the percentage free T4 (0.046-0.06 %), free T4 (1.26$\pm$ 2.03 ng/100 ml), total T3 (39-44 ng/100 ml) percentage free T3 (0.521-0.638 %) or free T3 (0.221 $\pm$ 0.26 ng/100 ml). Reverse T3 was undetectable in adult male, non-pregnant female and pregnant female serum. At 45 days of gestation fetal plasma T4 levels were similar and fetal T3 levels lower than in maternal serum but rT3 was detectable in fetal serum. These authors only looked at one other fetal group spread about an average fetal age of 62 days (range 57-65 days). Fetal plasma T4 had increased from 2.5 $\pm$ 0.3 to 4.3 $\pm$ 1.3 µg/100 ml (Mean $\pm$ S.D.; $P < 0.001$), fetal serum T3 remained unchanged and fetal rT3 increased from 5.2 $\pm$ 3.3 to 25.0 $\pm$ 11.4 ng/100 ml ($P < 0.01$). In this near-term fetal serum total and free T4 and total rT3 concentrations were significantly higher and total and free T3 concentrations significantly lower than corresponding values in maternal serum.

The Importance of the Fetal Thyroid in Development

Thyroid hormones exert a considerable effect on fetal growth and tissue differentiation of all mammalian species. Nathanielsz (1976) defines growth as:- "Any permanent increase in size resulting from an increase either in mean cell size or in the number of cells as a result of mitosis". Differentiation is the development of the specific characteristics of different tissues from initial cell lines all of which have the same genetic material. The development phases which occur in utero in the human and sheep are similar to those taking place in utero in the guinea pig. All available data indicate that the thyroid of the guinea pig fetus is capable of functioning independently of that of the mother and that maternal to fetal transfer of thyroid hormones at physiological concentrations occurs only to a small extent. Therefore fetal thyroid function is necessary for normal growth in utero.

In sheep the best study of the role of the thyroid hormones on body growth and development is that of Hopkins and Thorburn (1972) and Thorburn and Hopkins (1973) in the thyroidectomised fetal lamb. Thyroidectomy at 80-96 days of gestation resulted in reduced body weight at delivery. The major effects observed were shortening of the limbs and clear abnormalities in the development of the bones with retardation of ossification in the epiphyseal centres of the bone shaft. Becks, Scow, Simpson, Asling, Lie, and Evans (1950) reported that thyroidectomy of the neonatal rat resulted in effects on longitudinal bone growth, delay in appearance of ossification centres and slowed development of the bones of the skull. The effects of fetal thyroidectomy of the rhesus monkey were similar to those seen in the fetal lamb; reduction in the length of the long bones and a delay in osseous maturation with some changes in the facial characteristics, the maxillae were broader and the tongue was unusually prominent (Kerr, Tyson, Allen, Wallace and Scheffler, 1972). Radioidide damage to the fetal thyroid may occur as a result of ^{131}I treatment during pregnancy. In such an individual Pfannenstiel, Andrews and Brown (1965) reported that at eight months of age the neonatal bone

age was less than three months and body weight was low. This infant suffered from lethargy, hoarseness, frequent respiratory infection and low serum protein bound iodine (PBI).

Lambs that are thyroidectomised relatively early in gestation fail to stand, suckle, maintain a normal rectal temperature or establish normal breathing and die soon after birth. Thyroid hormones may be required for the synthesis of pulmonary surfactant. Wu, Kikkawa, Orzalesi, Motoyama, Kaibara, Zigas and Cook (1973) reported that injection of thyroxine into fetal rabbits accelerated the appearance of pulmonary surfactant. In sheep Avery (1975) has suggested that the critical period for the development of surfactant is 120-130 days and that glucocorticoids play an important role in this process. Fetal lambs thyroidectomised at 130 days of gestation survive for at least ten days post partum and do not show respiratory distress (Nathanielsz, Thomas and Jack unpublished observations reported in Nathanielsz, 1975).

A clear effect of thyroid hormones on the morphogenesis of the CNS in the rat has been shown in the cerebellar and cerebral cortex. The degree of the impairment produced by thyroidectomy of the newborn rat decreased with increasing age at thyroidectomy (Eayrs, 1960). Kerr et al, (1972) have reported a reduction in weight of the spleen, liver, pancreas, adrenal and placenta in thyroidectomised fetal monkeys.

Recently some evidence has been presented which suggests that placental insufficiency could explain the intra-uterine growth retardation and low concentration of thyroid hormones in the plasma of animals of low body weight. In the rat Wrutniak and Cabello (1983) observed a positive relationship between plasma thyroxine and body weight in fetal and newborn rats. In newborn lambs a positive relationship has been observed between plasma levels of T3 and T4 and birth weight (Cabello and Levieux, 1980). In guinea pigs the changes in the endocrine state of the growth retarded fetus has been studied by Jones et al, (1985) using uterine artery ligation to retard fetal growth. They reported that growth retarded fetuses exist in an

entirely different endocrine state from the normal. They found a delay in the developmental changes in the fetal plasma thyroid and adrenal hormone concentrations, a slow rate of fetal organ maturation and differential effects on organ growth. No differences were found in growth hormone concentrations between the normal and the growth retarded fetuses.

Effects of PTU on Experimental Animals

The weight of the thyroid glands in newborn guinea pigs born to mothers fed PTU 16-20 days or 21-38 days before birth were increased 13 and 50 times respectively. No significant thyroid hyperplasia was found in the mothers unless PTU treatment was prolonged. Daily administration of PTU either orally (10 mg) or by I.P. injection (5 mg) for a period of 12 to 14 days failed to induce goitres in normal newborn guinea pigs. Only minimal histological changes occurred in the thyroid gland (D'Angelo, 1967). Following daily treatment with 0.1 % PTU mixed into the food throughout pregnancy it was found that gestation was prolonged averaging 69.9 days in treated females compared with 67.9 days in normal animals. The females receiving PTU delivered 5 stillborn litters and 17 living offspring. The stillborn offspring had easily palpable thyroids. The average weight of those removed from 12 newborn animals was 2462 mg compared with 24 mg in normal newborn. Microscopically the follicles were lined with columnar epithelium and colloid was absent. There was a gradual regression in size of the thyroid gland in the neonate which began soon after birth although involution to normal size was not complete within eight and a half months. The goitrous male and female newborn showed no retardation in any phase of their later sexual maturation (Webster and Young, 1948; Webster, 1949).

Ortiz (1959) induced fetal goitres by feeding the pregnant guinea pig 50 mg PTU daily from about 40 to 100 days prior to mating. In the fetuses at 45 days the thyroid gland weight had increased three fold and a ten fold increase in weight was seen by 60 days. There was an increase of 140 % in fetal pituitary weight at 45 days of gestation in the fetuses of those mothers who had received PTU for longer. There were no significant effects of the drug on the weight of any other fetal organs. Ortiz also noted that the adrenal weights of untreated female fetuses were significantly greater than those of untreated males. Logothetopoulos and Scott (1956) found the thyroids of 30 day old guinea pig fetuses of mothers treated with PTU 15-30 days before mating were of the same weight and histological appearance as the

glands of control fetuses while at 36 days fetuses from treated animals showed increased thyroid weight and loss of colloid and there was a marked increase in cell height. The growth rate of fetal goitres was many times greater than that of corresponding maternal goitres in 43 and 60 day old fetuses. Expansion and folding of the follicular walls rather than increased follicle formation characterised the fetal goitres. When male and female guinea pigs were given 100 mg PTU daily for 30 days by stomach tube there was an increase in thyroid weight/body weight ratio in the females but not in the male guinea pigs although the glands in both cases were markedly hyperplastic. Pituitary, adrenal, kidney and gonad weights in relation to body weight were not changed but liver hypertrophy was sometimes obtained (Azarnoff and Leathem, 1948).

PTU given to pregnant rats on day 18 or 19 of gestation induced statistically significant increases in the weight of the fetal thyroid and in the ratio of fetal thyroid weight to body weight. There was a significant decrease in fetal body weight. Treatment of pregnant rats with 50 mg PTU daily from day 12 to day 16 of gestation resulted in the retardation of growth and in the degree of ossification seen in fetuses on day 17 of gestation (Eguchi, Fukiishi and Hasegawa, 1980).

Lascelles and Setchell (1959) treated pregnant ewes with methyl thiouracil (MTU). They found a significant ($P < 0.001$) increase in the length of the gestation period in the treated group and the administration of MTU resulted in the occurrence of goitre in the lambs. There was a significant decrease in the body length of the treated lambs and a decrease in body weight although the latter was not significant. Enlarged thyroid glands were obtained in the ewes which had been treated with MTU. A retardation of osseous growth and maturity was seen in the fetal lambs.

Postnatal Thyroid Hormone Concentration

In the first days of extra uterine life rapid changes occur in the level of function of the pituitary-thyroid axis in many species. The serum thyroxine level of neonatal guinea pigs increased to about four times the prenatal levels approximately 14 µg/100 ml being reached by the second hour post partum. From the second postnatal day onward serum thyroxine levels decreased coming down to the adult level (2.01 µg/100 ml) some time between day 21 and 42 (Pale et al, 1973). In humans normal infants have serum T4 concentrations of 7.8-16 µg/100 ml, T3 concentrations of 14-86 ng/100 ml and rT3 and TSH concentrations of 49-253 ng/ml and 0.5-18.5 µU/ml respectively (Van Herle, Young, Fisher, Uller and Brinkman, 1975). Free thyroxine levels are 4.1-7.2 ng/100 ml (Fisher and Dussault, 1975). The normal range of the adult plasma thyroid hormone concentrations are :- T4, 4-11 µg/100 ml; T3, 70-180 ng/100 ml; rT3, 25-80 ng/100 ml (Refetoff, 1979).

In pigs plasma thyroxine concentrations rose significantly from fetal values of 7.9 ± 0.3 µg/100 ml to 12.2 ± 0.5 µg/100 ml in the first 2.5 hours after birth. Plasma thyroxine levels averaged 7.2 ± 0.5 µg/100 ml at 11 days of age. The pattern of plasma T3 concentrations was similar to that of T4. Immediately after birth plasma T3 concentration were low (58.6 ± 3.8 ng/100 ml), they rose to 172 ± 17.9 ng/100 ml at 2.5 hours after birth and continued to rise to 214 ±18.3 ng/100 ml at 9 days after birth (Colenbrander et al, 1979). Serum T4, T3 and rT3 from 1 to 21 days after birth were also measured by Slebodzinski, Nowak and Zamystowska (1981). They observed the highest T4 levels to occur between birth and 36 hours. The T4 concentration at this time ranged from 7.8 ± 2.6 to 10.2 ± 1.25 µg/100 ml. There was a steady fall in T4 to a low value at about day 5 followed by a progressive rise at days 10 to 14. The pattern of T3 concentration was similar to that of T4 with the difference that there was a more pronounced rise immediately after birth to reach the highest level within 36 hours. (The average values ranged from 97.6 ± 10.66 at birth to 179 ± 13.87 ng/100 ml at 36 hours) There

was a second rise in T3 with a peak between days 3 and 7. By the end of 21 days the mean T3 and T4 concentrations were about half of the postnatal maximal level. The changes in rT3 concentrations resembled those for T4 and T3 during the first 24 hours of life. Later a progressive decrease occurred with a nadir at about day 3. This was folowed by a gradual rise to a relatively high and stable level during the third week of life.

In the foal the mean serum total T3 concentration increased to 991 ng/100 ml immediately after birth and was 935 ng/100 ml at day 4. Adult levels of 78 ng/100 ml were reached by 3 months of age. Total T4 concentration in the serum of foals was 28.90 µg/100 ml immediately after birth. By 4 days it had decreased to 11.2 µg/100 ml and it reached the adult levels by day 12 to 16 of life. Serum free T4 concentration at birth was 12.2 ng/100 ml by day 4 it had decreased to 5.9 ng/100 ml. The serum free T4 concentration in the adult was 2.6 ng/100 ml (Irvine and Evans, 1975).

In the calf the plasma T4 level is elevated in the first 3 hours of extra uterine life when it is 12.8 ± 0.6 µg/100 ml (Nathanielsz, 1969). There then follows a rapid fall in plasma T4 to a level of 4.2 ± 0.6 µg/100 ml on day 4. A second rise occurs with a peak value of 5.7 ± 1.2 ug/100 ml on day 21. The T3 concentration rises to a maximum level of 5 ng/ml on the first day of extra-uterine life and falls again to 2 ng/ml on day 5 (Nathanielsz and Thomas, 1973). Kahl, Wrenn and Bitman (1977) have studied the level of thyroid hormones in male and female calves. Immediately after birth the T4 and T3 plasma concentrations increased to 140 ug/l and 5.48 ug/l respectively they then fell rapidly to concentrations of 35.5 ug/l (T4) and 1.0 ug/l (T3). In the second and third week of life a small non-significant rise in T3 and T4 was found this was followed by a gradual decrease in T3 that reached a nadir at about 6 weeks of age. For T4 the lowest values were found at 6 weeks in female and at 8 weeks in males. No differences in T3 or T4 were seen between male and female calves during the first 4 weeks of age. After 6 weeks T3 gradually increased in both sexes. The normal adult T4 value, obtained at 18 weeks of age

in male calves, was 57 µg/l and in the female 48 µg/l. T3 levels were 1.4 ± 0.04 in male and 1.39 ± 0.11 µg/l in females. In newborn sheep the plasma T4 level immediately after birth is 18.2 ± 2.0 µg/100 ml by 5 hours it has decreased to 12.3 ± 1.1 and after 48 hours it is 10.4 ± 1.7. It then remains almost constant until day 61 of life (Nathanielsz, 1969a). In the lamb plasma T3 concentration changes abruptly. Within the first 3 hours after birth, it had risen to a mean level of 1.8 ng/ml. By the end of the first day of neonatal life plasma T3 measured 3.3 ng/ml and the T3 concentration continued to rise during the period in which T4 concentrations fell (Nathanielsz, Silver, Comline 1973c). Serum rT3 in 3 newborn lambs during the first 6 hours of postnatal life showed no consistent changes in concentration during this period but there was a gradual decrease in serum rT3 level during the first 3-4 days of life and at 42-90 days of age the serum rT3 concentration was comparable to that of adult sheep (Chopra, Sack and Fisher, 1975). Cabello and Levieux (1980) have measured the plasma T3 and T4 concentration in lambs. Plasma T3 concentration increased sharply between birth (2.78 ± 0.38 ng/ml) and 8 hours (4.73 ± 0.59 ng/ml). Then they declined until 16 hours (2.10 ± 0.1 ng/ml). A sharp increase was found between 16 and 44 hours (4.59 ± 0.48 ng/ml) and then T3 concentrations declined until day 5 (2.9 ± 0.1 ng/ml). A decrease in plasma T4 level was measured between 8 hours (96.6 ± 8.2 ng/ml) and 16 hours (74.8 ± 6.2 ng/ml) it then rose until 44 hours (113.6 ± 12.5 ng/ml) and subsequently decreased from 44 hours to 9 days (63.8 ± 6.1 ng/ml). After that an increase was apparent between 9 and 30 days (91.0 ± 7.3 ng/ml). The T4/T3 ratio increased sharply between birth and 18 hours after which it decreased until 20 hours. A further increase occurred between 20 hours and 20 days.

Thyroid Function in the Newborn

The availability of T4 and T3 post-natally coupled with the activation of non-shivering thermogenesis mechanisms are obviously important in permitting the newborn animal to maintain a stable body temperature. The newborn pig which has no brown adipose tissue differs markedly in this respect from the newborn of most other species e.g. rats, rabbits, guinea pigs, human infants, lambs, all of which are well supplied with brown adipose tissue (Hull 1966; Smith and Horwitz, 1965). Therefore the newborn pig is the most cold sensitive among neonatal domestic species. Its metabolic rate increases during the first postnatal day with a maximum on the second or third day. Marked changes in thyroid activity and the highest level of free thyroxine occur in the pig from 6 to 12 hours after birth. The well known thermogenic action of thyroid hormones led to the suggestion that thyroxine might be a mediator of the postnatal stimulation of thermogenesis in the newborn pig (Kaciuba-Uscilko, 1972; Slebodzinski, 1979). Newborn lambs thyroidectomised at 130 days of gestation are unable to maintain a normal rectal temperature, fail to gain weight and die within about 10 days (Thomas and Nathanielsz, 1982). Thyroidectomy of the calf during the second week post-partum results in a considerbale diminution in growth rate (Nathanielsz, 1970). In a human infant radioiodine damage to the fetal thyroid resulted in a hypothyroid child who was unable to develop, gaining neither weight nor height. After thyroid replacement therapy the child was doing better with height, weight and bone age almost normal (Pfannenstiel et al, 1965).

Cabello and Levieux (1980) induced the birth of six lambs 7 days before term by injection of oestrogen into the ewes. Thyroid function seemed to be affected by their prematurity during the first month of neonatal life. The conversion of T4 to T3 was reduced and plasma T3 concentrations were signficantly lower in the preterm animals from 1 hour to 30 days after birth. The thyroid glands in these animals seemed to be relatively insensitive to a TSH surge induced for example by cold and their thermoregulation was impaired. These workers

concluded that the abnormalities observed in these animals could partly explain the high mortality rate observed in premature mammals. In the preterm infant Schonberger, Grimm, Emmrich and Gempp (1979) have emphasised that thyroid hormone injections lower mortality. In lambs it seems that thyroid hormones regulate body temperature (Fisher and Odell, 1969) and stimulate the synthesis of pulmonary surfactant (Erenberg, Rhodes, Weinstein and Kennedy, 1979). Thus during the first hours of postnatal life T4 and T3 play a major role in determining survival of the neonate.

Body Weight

a. Fetal weights

The term growth can have different meanings, e.g. weight gain, head enlargement, length increase and sometimes, incorrectly, to include organ differentiation. The rate of growth before birth, like the rate of growth afterwards, depends primarily upon the supply of food and the ability of the fetus to take in and make use of the food. The maternal and fetal placental circulation and the concentration of nutrients in the maternal-fetal transfer are important. The growth in weight of the fetus is not uniform but becomes more and more rapid as gestation advances (Wigglesworth, 1966). In man the high rate of growth occurs before about the 36th week of gestation when the mean weight of the fetus is about 3 Kg after this the growth rate begins to fall off (Widdowson, 1968). In macaque, variation in body weight of the fetuses at each age is minimal and there is good correlation between body weight and gestational age throughout gestation. The fetal body weight at 50 days of gestation is 3.78 g increasing to 93.3 g at 100 days and to 436 g at 150 days (Pickering and Kontaxis, 1968). In the fetal mouse the average weight of entire litters at 12 days of gestation was 97.4 mg and it increased to 154.4 mg at 13 days, to 582.7 mg at 16 days and to 1278 mg at 18 days. Between the 17th and 18th days the rate of increase declined (Jacobson and Brent, 1959).

In sheep data from fetuses of single and twin pregnancies are treated separately because of the differences in weight. The mean weight of the single fetuses increased from 3010 $\pm$ 368 at 115 to 119 days to 4509 $\pm$ 1107 at 140 to 144 days. The corresponding weights of the twin fetuses for these gestational ages were 2340 $\pm$ 268 g and 3754 $\pm$ 1151 g respectively. The mean weights of all the placentae at term was 334 $\pm$ 22 g. The placentae of the single fetuses are heavier (412 $\pm$ 53g) compared to those of the twin fetuses (305 $\pm$ 18g) (Robinson, Kingston, Jones and Thorburn, 1979). In horses, the mean weight of the fetal horse was increased from 7.0 Kg at 4-5 months to

20.9 Kg at 7-8 months. At birth the mean fetal body weight was 44.8 Kg (Platt, 1984).

b. Newborn weights

At birth the guinea pig is a "going concern" able to see, to walk and to maintain its body temperature with the aid of a well developed coat. Guinea pigs are born in a mature state with a full set of teeth. They are normally nursed but can survive without any milk at all (Harris, 1936 & 1937; Moog, 1979). The newborn rat is born blind, helpless, hairless and entirely dependent on the mothers milk for three weeks. The newborn rat is born in an immature state after a comparatively short period of gestation. Birth takes place before the growth curve reaches its steepest part and there is no measurable break in the curve at the time of birth (Widdowson, 1968).

The newborn lamb differs from the human infant or the calf in that it exhibits little or no post-natal weight loss. In the group of lambs which was studied by Nathanielsz (1969a) weight gain occurred steadily from day one. The newborn lambs average body weight was 2.7 Kg and it had increased to 3.05 Kg on day 2. The newborn calf does not gain weight for a considerable time after birth. The male newborn calf weighs 43 $\pm$ 2 Kg immediately after birth while the female body weight was 39 $\pm$ 2 Kg at this time. In the second week of life the male body weight was 43 $\pm$ 2 Kg and the female was 44 $\pm$ 2 Kg. At the end of the fourth postnatal week the male and female average body weights were 46 $\pm$ 2 Kg and 47 $\pm$ 2 Kg respectively (Kahl et al, 1977).

The main conclusions of this introduction are :-

a) Earlier studies on the function of the fetal thyroid in small laboratory animals have been limited to observations in the rat and rabbit . It has been difficult to demonstrate any marked effect of thyroid deficiency in fetal life. In both these species the fetal thyroid starts functioning relatively late in gestation and the young leave the uterus before reaching the stages of development for which thyroid hormones are required.

b) In many other species, including sheep and man, the fetal thyroid starts functioning early in gestation and the young are born in a more mature state. In these species the fetus may be more susceptible to thyroid hormone deficiency.

Investigation of human gestational phenomena are limited. Some data may be obtained from abortion, premature delivery and post natal cord bloood samples from term deliveries. These data must be treated with caution since they are affected by anaesthesia or delivery stress. There are some data from fetal sheep studies. but there are several differences between the physiology of gestation in the sheep, rat and human (see table overleaf).

	Sheep	Human	Guinea Pig	Rat
Fetus Mature at birth	Yes	Yes	Yes	No
Maturation late in gestation	Yes	NO	No	No
Fetal cortisol causes birth	Yes	No	No	No
Fetal steroid causes maturation	Yes	Yes	Yes	Yes
Syndesmochorial placenta	Yes	No	No	No
Haemomonochorial placenta	No	Yes	Yes	Yes
Ruminant	Yes	No	No	No
Steroid crosses the placenta	NO	Some	Some	Easily
T4 and T3 cross the placenta	No	a Little	a Little	Easily
Main steroid cortisol	Yes	Yes	Yes	No

Surprisingly, no attention has been given to the thyroid hormone inter-relationships between the fetus and mother in the guinea pig. The aim of this study was to quantify plasma thyroid and adrenal hormone concentrations in both pregnant guinea pigs and their fetuses and to study abnormal thyroid function during pregnancy by eliminating fetal sources of thyroid hormone. Thus it was intended to determine the effects of thyroid deficiency in the fetus and perhaps gain a better understanding of the physiological role and the development of the thyroid gland during this period.

MATERIALS AND METHODS

Materials and methods

Care of the animals

Dunkin Hartley guinea pigs of both sexes were used throughout this study. Animals were obtained from the closed colony maintained in the Animal House of the university and were housed, 2 to 3 animals per cage, under temperature controlled conditions and fed pelleted commercial diet and water ad libitum. All animals were allowed to feed normally up to the time of experimentation.

Experimental animals

The experiments were carried out on three groups of animals :-

I Pregnant guinea pigs and their fetuses.

II Neonatal and young guinea pigs.

III Adult male and female guinea pigs.

1. Pregnant guinea pigs

The stage of pregnancy was determined by:-

(A) When the vagina was opened a female guinea pig was caged with a male and the day on which sperm was found in the vagina was taken as day 1 of pregnancy.

(B) Pregnant guinea pigs were caged with a male and the day of delivery was designated day 1 of pregnancy (female guinea pig enter oestrous and ovulate immediately after delivery).

(C) By palpation of the pelvic oulet of the pregnant guinea pigs for signs of relaxation, resulting in an increase in diameter, and indicating imminent parturition (Elvidge, 1972). The gestational age was verified by comparison with the fetal growth curve described by Draper (1920).

For the collection of blood samples and endocrine glands and tissues from pregnant guina pigs and their fetuses groups of pregnant guinea pigs at 35,37,40,45,47,50,55,60,62, and 64-67 days of pregnancy

(700-1200 g body weight) were either killed quickly by a blow at the back of the neck or anaesthetised by an I.P. injection of sodium pentobarbitone (45 mg/Kg body weight). The anaesthetic dose was administered in two halves, the first half given quickly after removing the animal from the cage . After 10 minutes 5 ml blood was collected from the mother by cardiac puncture then the other half dose was given. The fetuses were located by palpation and the abdomen and peritoneum were opened by a mid-line incision and the uterus was exposed. Blood was collected from the umbilical vessels of each fetus. After sampling the fetuses more blood was taken from the mother by cardiac puncture and she was then killed.

Endocrine glands were then quickly removed from mother and fetuses, weighed on an Oertling fine balance and prepared for histological or chemical study. The time between the first half-dose of anaesthetic and the killing of pregnant guinea pigs did not exceed 30 minutes whilst the time of collection of blood samples from guinea pigs killed by a blow to back of the neck did not exceed 4 to 6 minutes. All experiments were performed between 11.00 and 12.00 a.m..

Neonatal and young guinea pigs

Experiments were carried out on 110 male guinea pigs (60 to 250 g body weight). Anaesthesia was introduced by I.P. injection of sodium pentobarbitone as soon as possible after the animal was taken from the cage and blood samples were collected by cardiac puncture. Animals were sampled at exactly 1,4,9,24,30 and 48 hours after birth and additional samples were collected on day 3,4,6,9,14, and 21 days by cardiac puncture. Variation in size and age of neonates and young guinea pigs affected the volume of blood which it was possible to collect. The first 24 hours of life has been designated as day one.

The growth of 97 young from 26 litters ranging from day 1 to day 14 (the day of weaning in the animal house) was followed by daily weighing. The young were allowed to feed from their mothers ad libitum.

Adult guinea pigs

Guinea pigs of both sexes were used (700-900 g body weight). Guinea pigs were killed by a blow to the back of the neck. Blood samples (5 to 7 ml) were collected by cardiac puncture.

Treatment of samples

Blood samples were collected in EDTA tubes and centrifuged immediately. The plasma was aliquoted and stored at (-20° C) until assay. Immediately after killing the animals endocrine glands were removed, separated carefully from connective tissue and fat, weighed on an Oertling fine balance and prepared for histological study (fixed in 10 % formal saline) or chemical study (frozen at -20° C).

Histology

Methods

After the glands and tissues were fixed in 10% formal saline and prior to embedding, the fixed material was washed to remove excess fixative and then dehydrated by passing it through increasing strengths of alcohol. Thus the tissues were passed through 50, 70 and 90 % alcohol and then passed through two separate baths of absolute alcohol, (alcohol I and II), each stage lasting for two hours. The tissues were then placed in inhibisol (1,1,1, Trichloroethane) overnight to remove the dehydrating agent. After that the tissue were passed through melted paraffin wax then the dehydrated tissues were allowed to steep in melted paraffin wax baths, I, II and III for two hours each. (Before dehydration, clearing and embedding of the samples of bone they were first decalcified by soaking for several weeks in formic acid). Trapped air in the specimens was removed at reduced pressure. Reduced pressure was obtained using the vacuum embedding oven (Townson and Mercer Ltd, Croydon England). Then the paraffin wax was cooled and became solid forming a block which could be cut. Section thickness varied from 4 to 6 μ. Slices of this thickness were cut using a Spencer 820 microtome.

Staining

After the sections were cut they were mounted on glass slides and thoroughly dried before staining. In this study Haemotoxylin and Eosin (H & E) stain was used for thyroid, adrenals, skin, bone and pituitary; Mallory-Heidenhain (M & H) stain was used, as well, for the pituitary.

a. Protocol for H & E :-

1. Section to Inhibisol for 3 minutes.
2. Rinse in alcohol as follows

A	Absolute alcohol I	for	2 minutes
B	Absolute alcohol II	for	2 minutes
C	90 % alcohol	for	2 minutes
D	70 % alcohol	for	2 minutes

3. Running tap water for 30-60 seconds
4. Weigert's Haemotoxylin for 10-15 minutes
5. Running tap water for 1-2 minutes
6. Differentiate in Acid-Alcohol for 5-10 seconds
7. Running tap water for 5 seconds
8. SCOTTS (see below) for 30 secs
9. Running tap water for 5 seconds
10. Eosin for 1-2 minutes
11. Running tap water for 30-60 seconds
12. Rapid dehydration with 70%, 90% absolute alcohol I and absolute alcohol II for 30 seconds.
13. Inhibisol for 2-3 minutes
14. Sufficient DPX was applied just to cover the section, a cover slip was then applied to protect and preserve the section. Sections required several hours at room temperature to set before they were ready to examine.

(SCOTTS :-

Sodium bicarbonate	3.5 g
Magnesium sulphate	20 g
Thymol	1 small crystal
Tap water	1000 ml)

b. Protocol for M & H stain (Rapid one-step method)
Pituitary sections were stained with (M & H) according to the technique of Cason, (1950)

1. Sections to inhibisol for 3 minutes
2. Rinse in alcohol as follows

A	Absolute alcohol I	for	2 minutes
B	Absolute alcohol II	for	2 minutes

C	90 %	alcohol	for	2 minutes
D	70 %	alcohol	for	2 minutes

3. Running tap water for 30-60 seconds
4. Sections to Azan stain for 5 minutes
5. Running tap water for 3-5 secs
6. Rapid dehydration with 70 %, 90 % absolute alcohol I and absolute alcohol II.
7. Continue as in H & E stain protocol (above).

Mallory-Heidenhain stain :-

In 200 ml distilled water dissolve each of the following fully before adding the next compound.

1. 1 g phosphotungstic acid
2. 2 g orange G
3. 1 g aniline blue
4. 3 g acid fuchsin

Cortisol Assay Method

Solvents and Reagents

Buffer reagents (analar grade) were obtained from B.D.H. chemicals Ltd Poole England; dextran T-70 from Pharmacia; charcoal Norit GSX from Hopkins and Williams Ltd.; gelatin from Sigma; Freund's complete adjuvant from Miles Laboratories Inc. U.S.A.; ^{3}H cortisol from The Radiochemical Centre, Amersham, U.K.; ($^{4}_{\Delta}$-pregnen-11β,17α,21 triol-3-20-dione 3-CMO:BSA from Steraloids Ltd U.S.A.. Other steroids were obtained from Sigma Ltd with the exception of 17α OH progesterone and cortisone which were obtained from Koch Light laboratories Ltd, 11α OH progesterone from Aldrich Chemical Co U.S.A. and oestradiol from Organon Ltd.

Immunisation

Five New Zealand white rabbits were immunised using the following regime. Cortisol 3-CMO:BSA was emulsified in a mixture of Freund's complete adjuvant and 0.15 M NaCl (3:1 v/v) and a total volume of 2 ml (containing 1 mg) was injected at multiple sites subcutaneously on the back of each animal. Blood samples were collected from the marginal ear vein of each rabbit 21 days after the primary and 10 to 14 days after each subsequent immunisation. Preliminary assessment of antisera included a determination of cortisol antibody titre, an examination of cross-reactivity with steroids structurally related to cortisol, a demonstration of parallelism and an assessment of the recovery of the assay. Each parameter was checked using the assay system described below. The assay method was adapted from that of Fahmy, Reed and Hillier (1975) for the assay of cortisol in human serum.

Assay buffer and dextran charcoal

The assay buffer was 0.01 M NaCl (pH 7.4) containing gelatin (0.1 % w/v) prepared in distilled water. Dextran

charcoal suspension was prepared by dissolving 50 mg dextran T-70 in 200 ml assay buffer followed by the addition of 500 mg of charcoal. A fine suspension was obtained by mixing using a magnetic stirrer. This suspension was always prepared at least one hour before use and was continuously stirred over ice before it was used.

Standard cortisol solution

Cortisol was stored as a 100 µg/ml solution in ethanol at 4°C until required. A solution of 10 µCi/ml of (^{3}H) cortisol was prepared from the stock and stored at 4°C. This solution was diluted with assay buffer before each assay to give a final concentration of approximately 30,000 cpm per 100 µl.

Assay procedure

Standards were prepared by diluting the 100 µg/ml stock solution of cortisol in ethanol to give a series of tubes containing 0.5, 1, 5, 10, 50, 100, 500, 1000 ng/tube (in duplicate) in 100 µl of ethanol. A similar volume (100 µl) of ethanol was added to a further two tubes to act as a zero standard, allowing the measurement of the absolute amount of labelled cortisol bound to the antiserum in the absence of unlabelled material. Two tubes were used to check the efficiency of the removal of free cortisol from solution by the dextran charcoal (non specific binding). No antiserum was added to these tubes. A further two tubes were prepared containing only label and buffer. These tubes were used to assess the total counts in the assay system.

Aliquots of 100 µl of plasma were transferred to fresh tubes and diluted with ethanol (55 µl). The tubes were mixed on a vortex mixer, centrifuged at 4°C for 10 minutes at 2500 rpm and then 250 µl aliquots of the ethanolic supernatants were transferred into assay tubes.

The standard solution and the ethanolic

extracts of plasma samples were evaporated under compressed air at 40°C. Assay buffer (100 µl) was added to all tubes and they were then mixed on a vortex mixer for at least five seconds. The optimum dilution of antiserum was prepared in assay buffer and 100 µl was added to all tubes except the blank and the total tubes which received 100 µl of assay buffer instead. After mixing for 5 seconds on a vortex mixer the tubes were left to stand at room temperature for at least 30 minutes. ^{3}H cortisol solution (100 µl) was then added to all tubes and after vortexing briefly again the tubes were transferred to a constant temperature water bath at 30°C and incubated for a further hour. At the end of this incubation period the tubes were mixed briefly and left to stand in ice for 15 minutes then 0.5 ml of dextran-charcoal suspension was added to all tubes except the total tubes which received 0.5 ml of assay buffer. The tubes were then centrifuged for 10 minutes at 4°C and 2500 rpm. Finally the supernatants were decanted into scintillation vials and 10 ml of tritoscint was added. The vials were then transferred to a Beckman LS 7000 liquid scintillation counter and counted within a 2 % counting error at a tritium efficiency of 56 %. Each adrenal gland was homogenised in 0.5 ml normal saline and 0.1 ml of this homogenate was extracted with 2 ml of ethanol, centrifuged and the supernatant evaporated and assayed.

Calculation

A standard curve was constructed in the normal way by plotting the log of the amount of cold cortisol in the standard tubes on the abscissa and the counts bound on the ordinate. The amount of steroid present in the sample tubes was then read from the curve.

Validation

Twenty one days after the primary immunisation against cortisol plasma was obtained from the five rabbits. Fig. M1 shows the antibody dilution curve at zero cortisol concentration for

each rabbit. Rabbit E produced the best antiserum and was further immunised and bled after 14 days. Fig. M2 demonstrates the improvement in antibody titre and Fig. M3 illustrates a nine point full standard curve for antiserum dilutions of 1:500, 1:1000 and 1:10,000. As a result of this dilution 1:1000 was selected for future use.

The cross-rectivity of antiserum with steroids related to cortisol is indicated in Figs M4, M5 and M6. The cross-reactivities were assessed at concentrations of 1 ng, 10 ng, 100 ng and 1000 ng per assay tube for each steroid. The figures indicate that antibody E is able to distinguish cortisol from structurally related steroids at all concentrations.

In order to test for cross-reaction with other materials besides the steroids mentioned above the following procedure was used. Human plasma was diluted with assay buffer to obtain 12.5, 25, 50 and 100 % plasma. Aliquots of 100 µl of each dilution were extracted in triplicate with 0.5 ml of ethanol and centrifuged. Aliquots of 250 µl of the supernatants were placed in fresh tubes and evaporated to dryness at 40 °C under air. The standard curve for pure cortisol and the curve generated by plotting the volumes of plasma extracted on the abscissa are parallel (Fig M7) indicating that the antiserum is measuring only cortisol in the human plasma extracts.

Recovery

Finally, the recovery of the assay was estimated by extraction of human plasma samples containing added cortisol using the following procedure. The 100 µg/ml solution of cortisol was diluted in ethanol to give a series of tubes (in triplicate) containing 5, 10, 50 and 100 ng of cortisol in 1 ml of ethanol. After evaporation of these ethanolic solutions under compressed air at 40°C, 5 ml pooled human plasma was added to each tube, vortexed briefly and the tubes were then left at room temperature for 20

minutes for the cortisol to be fully taken up. Aliquots of 100 µl from each tube were transferred to fresh tubes, extracted with 500 µl of ethanol, centrifuged and 250 µl of the supernatant was transferred into assay tubes. The recovery was calculated from a linear regression drawn through the results of this assay plotted against the amounts of cortisol added and found to be 102.5 %(Fig-M8).

The intra assay coefficient of variation was determined using 20 determinations of a plasma sample and found to be 11.5 %.

Efficiency of cortisol extraction from guinea pig plasma

Samples were prepared by adding ^{3} H labelled cortisol to 100µl aliquots of plasma from ten different guinea pigs and extracting with 600µl of ethanol. As in the cortisol assay 200µl aliquots of these extracts were then taken and transferred to scintillation vials for evaporation and counting.

The extraction efficiency was found to be 105.6±4.1% (Mean ± SEM: n=10).

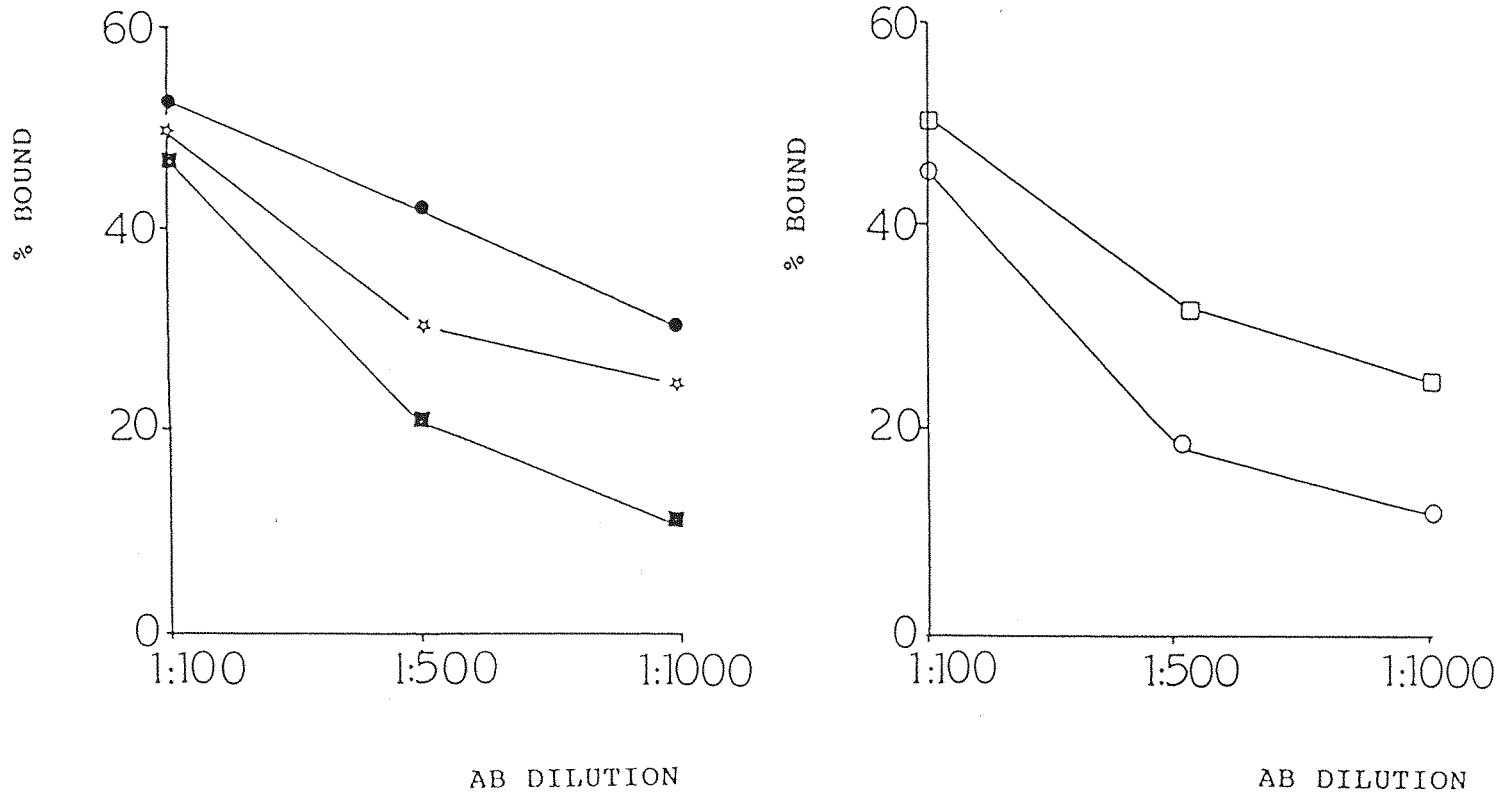


Figure M-1 Ab dilution curves at zero F concentration. Rabbit A (■), Rabbit B (☆), Rabbit C (○), Rabbit D (□), Rabbit E (●).

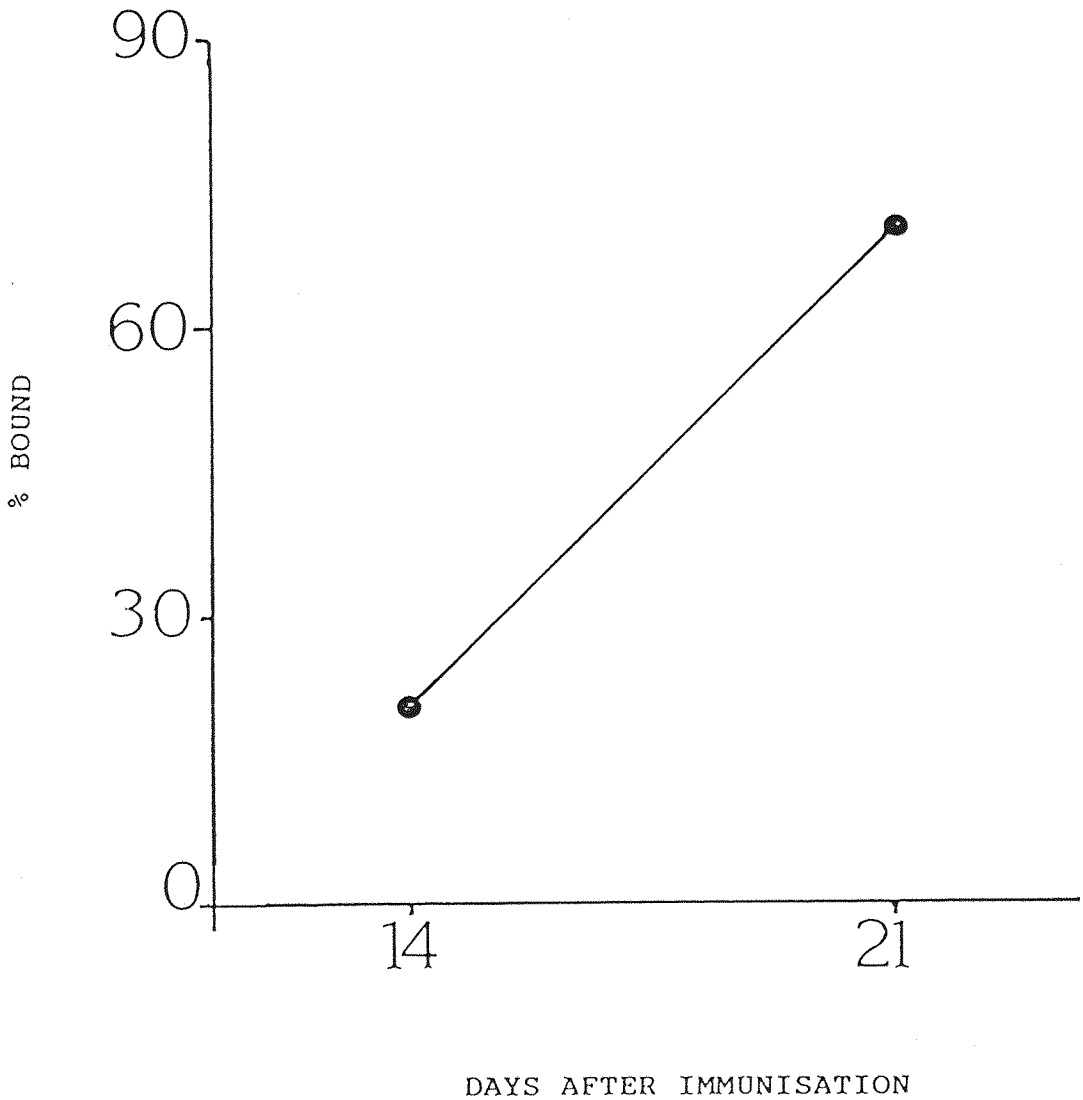


Figure M-2 Improvement of Ab binding at dilution 1:500 for rabbit E after the second immunisation.

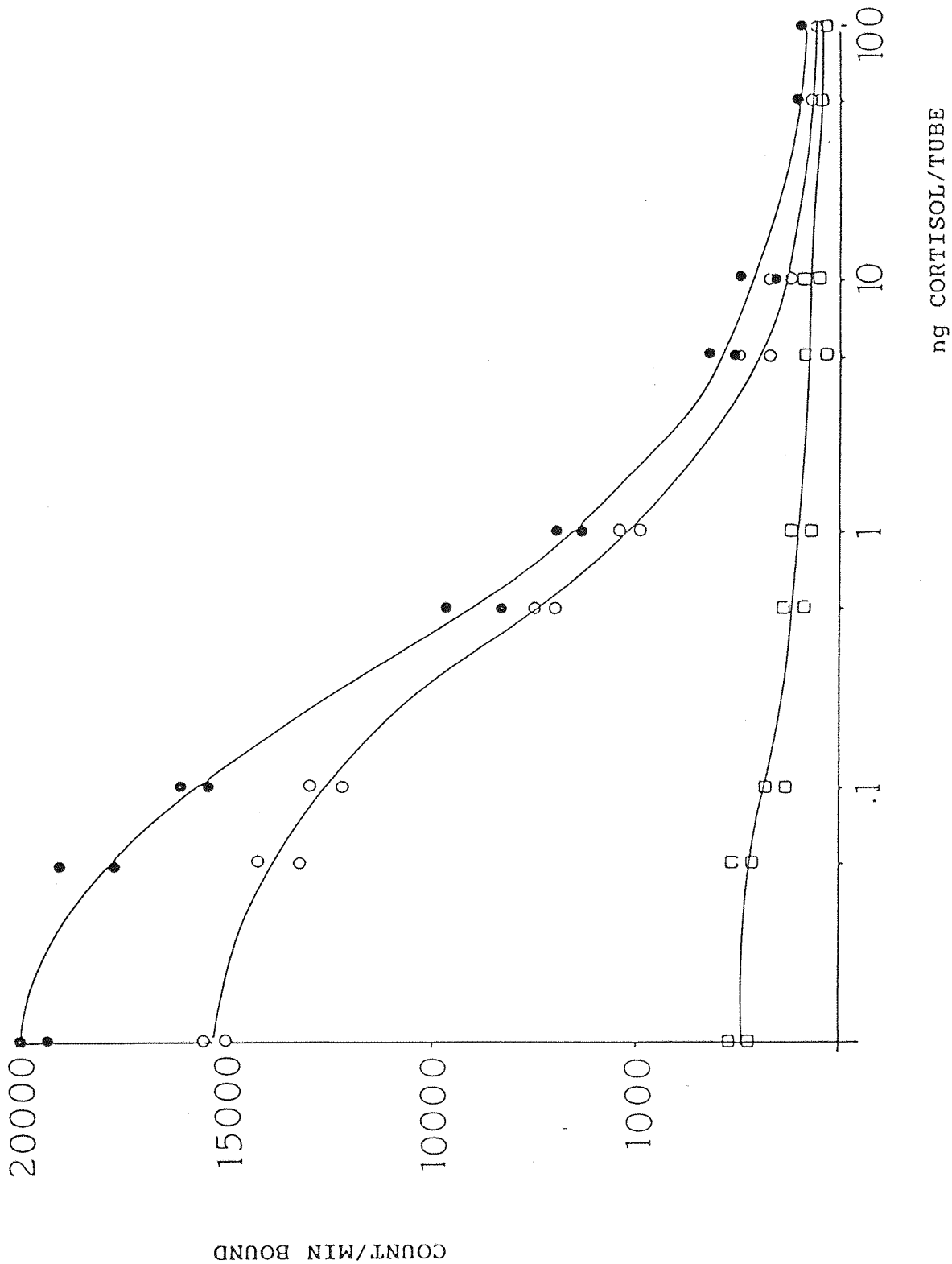


Figure M-3 Nine point standard curves at Ab dilution 1:10000 (□), 1:1000 (○), 1:500 (●).

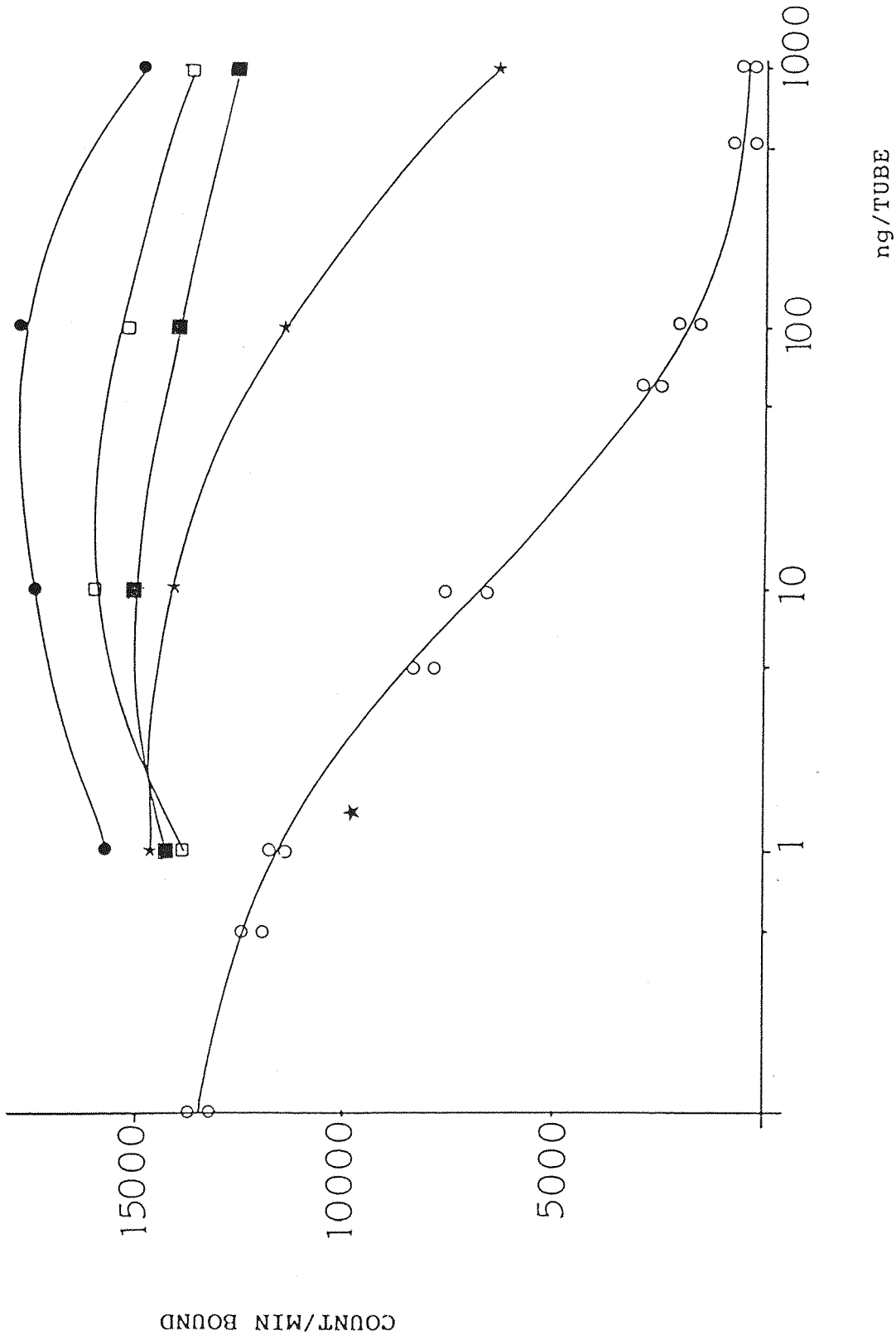


Figure M-4 The cross-reactivity of cortisol Ab with :-
Cortlcosterone (□), Deoxycortlcosterone (■),
Progesterone (★), Pregnenolone (●).

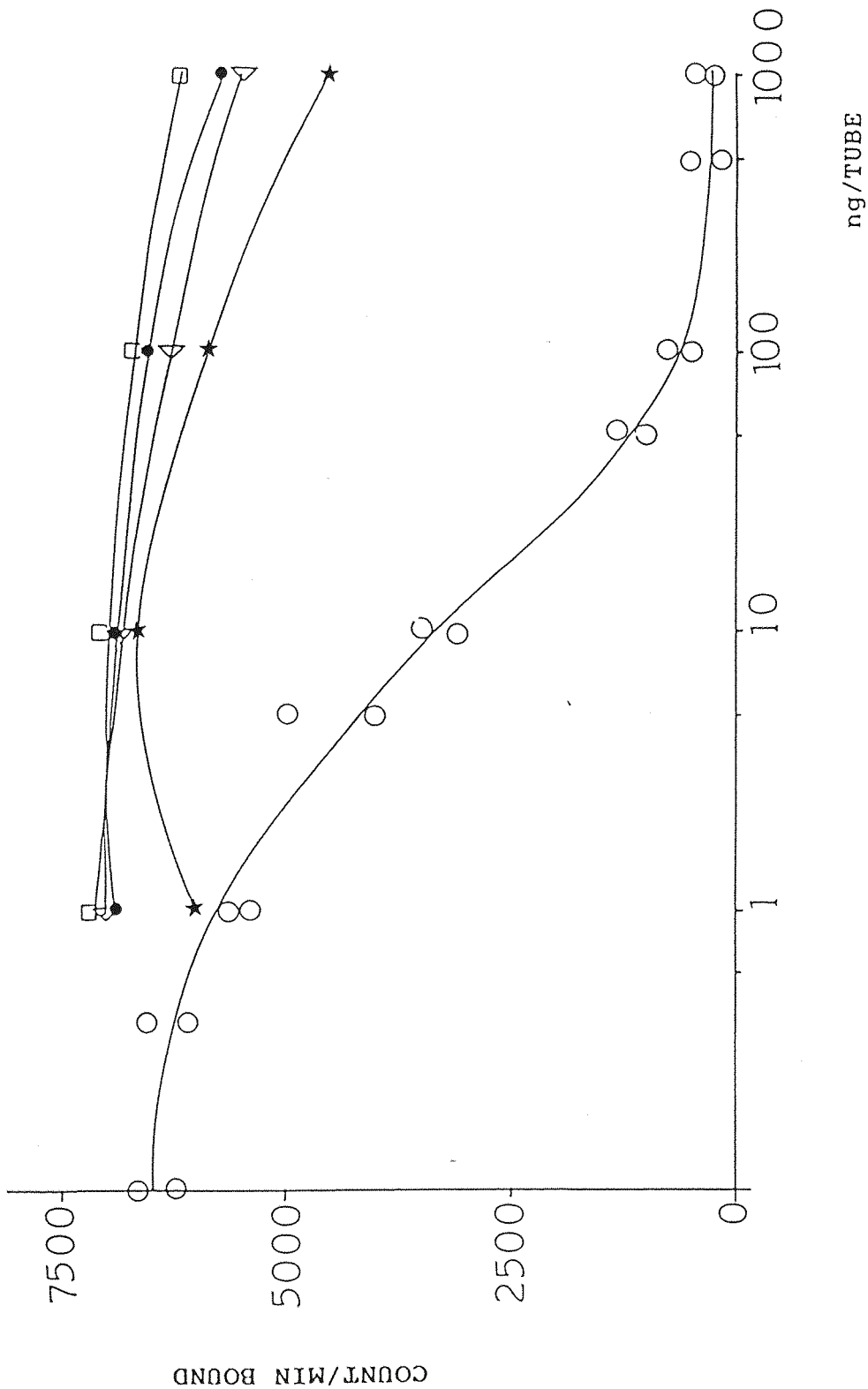


Figure M-5 The cross-reactivity of cortisol Ab with :-
11α-Hydroxy-Progesterone (□), 17α-Hydroxy-Progesterone
(●), 17α-Hydroxy-Pregnenolone (◁), Cortisone (★).

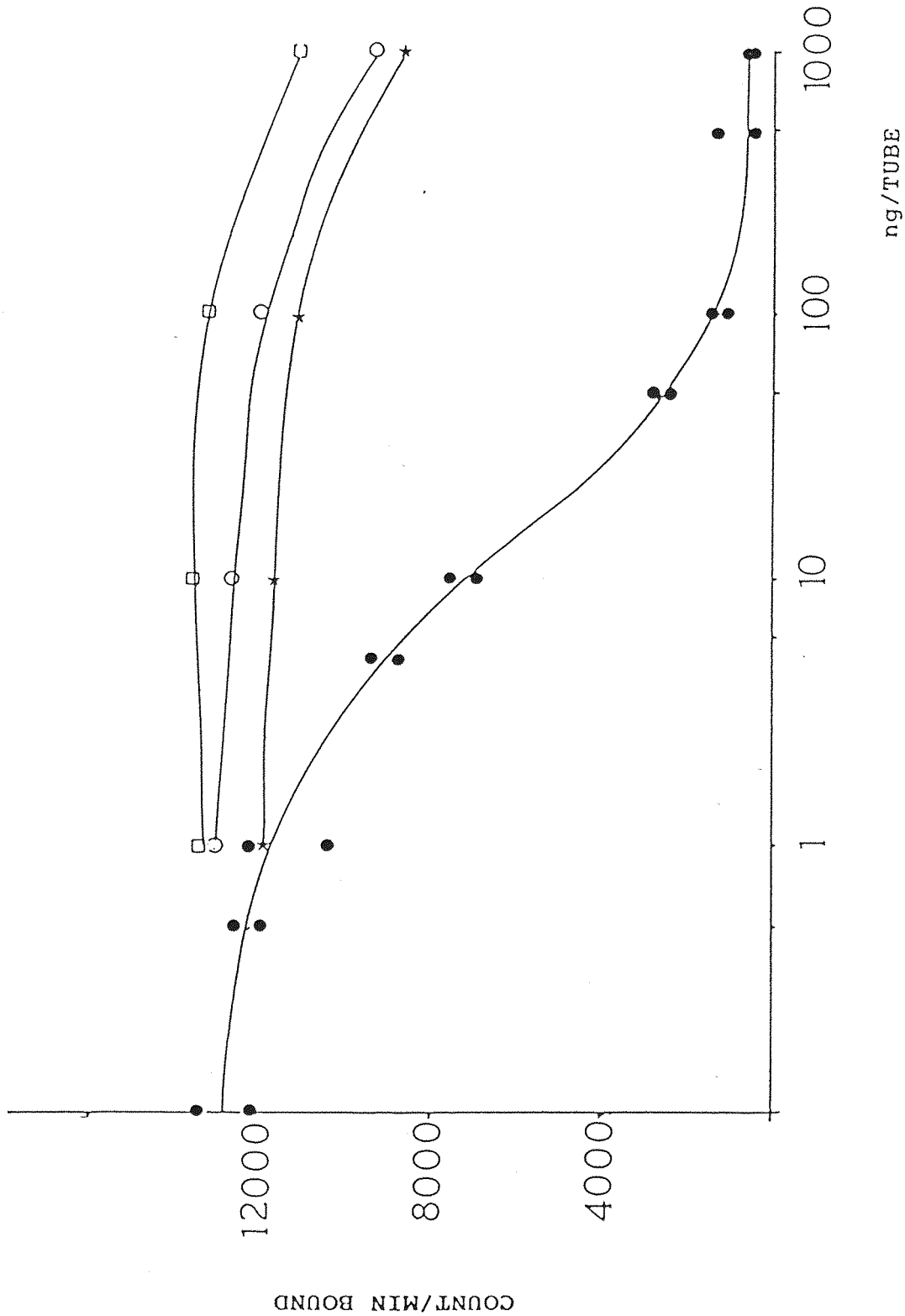


Figure M-6 The cross-reactivity of cortisol Ab with :-
Estrone (□), Testosterone (★), Estradiol (○).

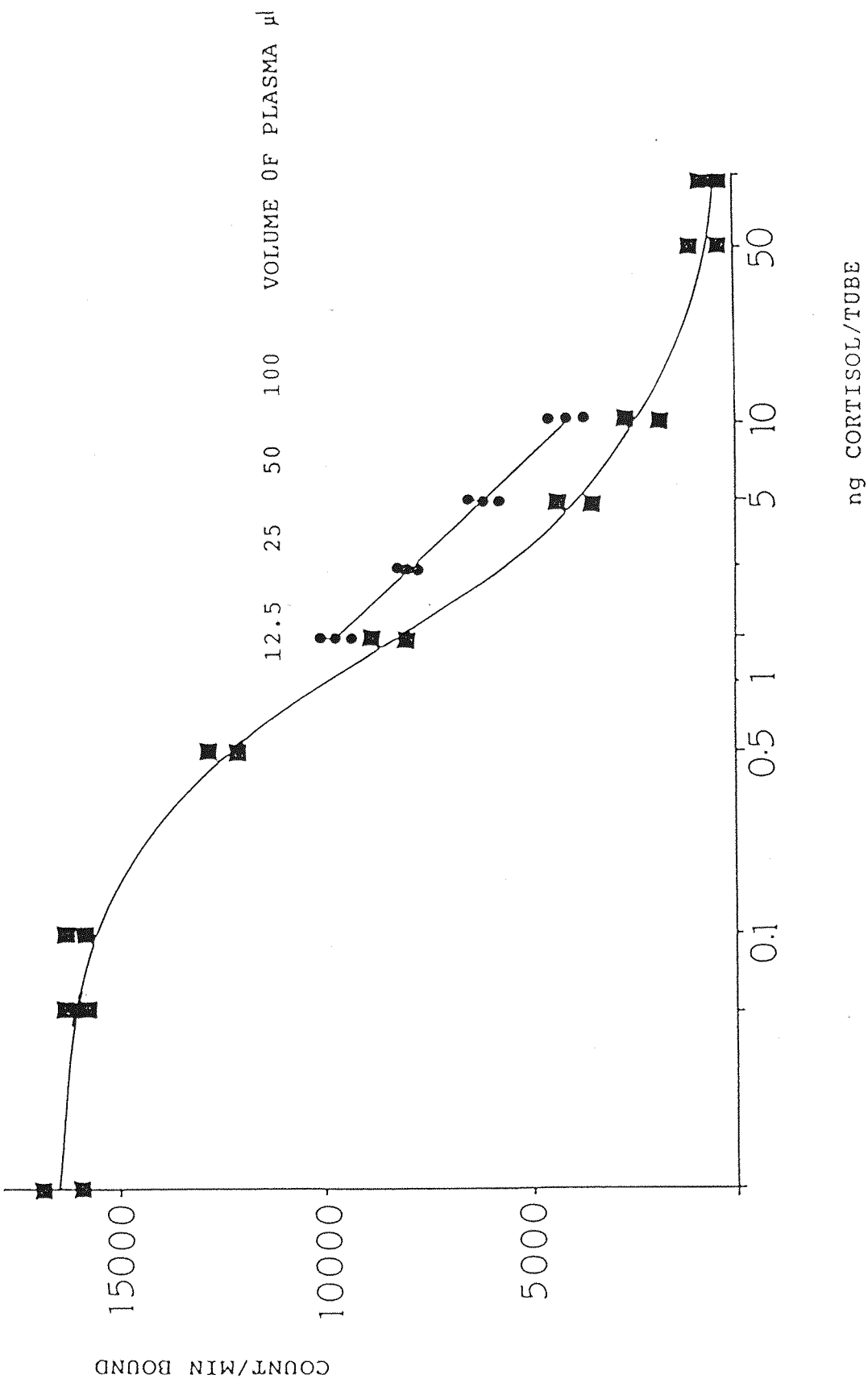


Figure M-7 Parallelism between the standard curve of Ab from rabbit E (■), and extracted plasma (●).

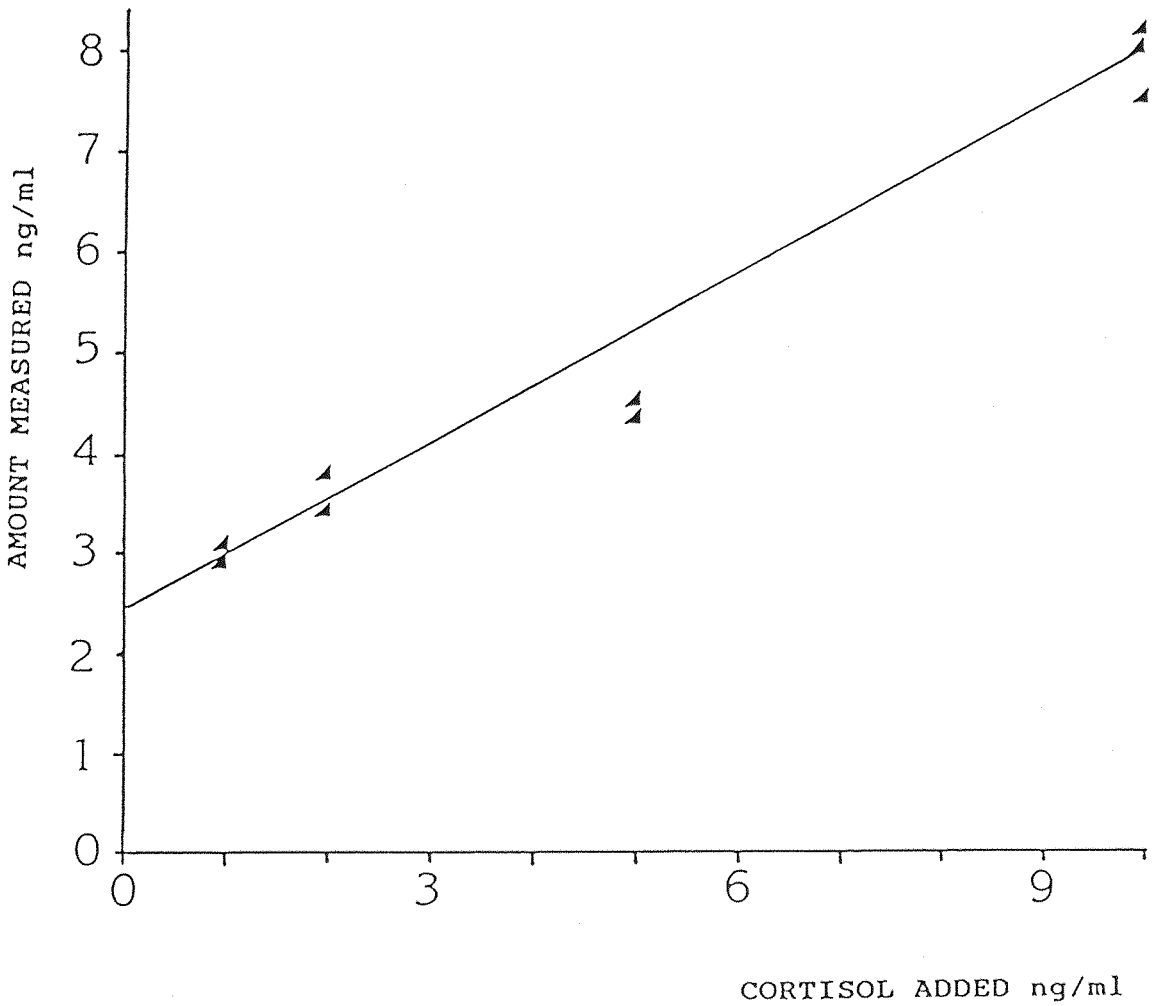


Figure M-8 Recovery of the cortisol assay.

Thyroid Hormone Assays

1. Competitive Protein Binding (CPB) assay for Thyroxine - T4

Plasma thyroxine concentrations were measured using an adaptation of the competitive protein binding assay of Murphy and Pattee (1964) as described by Thomas, Krane and Nathanielsz, (1978).

Solvents and reagents :-

Buffer reagents (analar grade) and Amberlite resin IRA 400 (Cl) standard grade were obtained from B.D.H. Chemicals Ltd. . Radioactive thyroxine (^{125}I - T4) was obtained from the Radiochemical Centre, Amersham, U.K. and thyroxine (sodium salt, pentahydrate) from Sigma.

Assay Buffer

One litre of barbital buffer (15.4 g sodium barbitone, 2.76 g barbituric acid-pH 8.6) was made up fresh in distilled water before each assay.

Binding solution

Radioactive solution containing human plasma was prepared as follows:- 20 µCi of ^{125}I-T4, 7.5 ml pooled human plasma, 2.5 ml phenol made up to 250 ml with barbital buffer and left to stand for at least 15 minutes before use.

Standard Solutions

Standard thyroxine solution (100 ug/ml) was made up by dissolving 11.029 mg of thyroxine (sodium salt pentahydrate) in 100 ml of ethanol. The solution was stored at 4°C in a plastic bottle until required.

Assay Procedure

Standards were prepared by diluting the 100 µg/ml solution of thyroxine in ethanol to give a series of tubes containing 0.5,1,2,5,7.5,10,15 and 20 ng/tube (in duplicate) each in 100 µl of ethanol. The same volume of ethanol was added to a further 3 tubes to form a zero standard. Plasma samples (200 µl) were extracted in duplicate with 2 ml ethanol, vortexed for 10 seconds and centrifuged for 10 minutes at 2,500 rpm . The ethanolic supernatants were decanted into assay tubes.

The standard solutions and the ethanolic extracts of plasma samples were evaporated to dryness under air at 40°C. Binding solution (1 ml) was added to each tube. After vortexing briefly the tubes were left to stand at room temperature for 10 minutes and then all tubes were transferred to a constant temperature waterbath for 5 minutes at 45°C. At the end of the incubation the tubes were placed on ice for 5 minutes. One spoon of resin was added to each tube before they were shaken for 2 minutes on a mechanical shaker (Griffin and George Ltd.) they were then returned to ice. Cold assay buffer (3 ml) was added to each tube and the resin was allowed to settle. Finally 1 ml of each supernatant was transferred to a counting vial. The vials were counted in a Beckman Biogamma or LKB Quatro Clinigamma counter. Fig M 9 shows a typical standard curve.

Thyroid glands were thawed and frozen 4 to 6 times just before homogenising in 3ml of n-butyl alcohol . The homogenate was centrifuged for 10 mintes at 2000 rpm and then 1 ml of the supernatant was evaporated down for thyroxine assay.

Recovery

The recovery of the assay was estimated by extracting and assaying human plasma containing added cold thyroxine. The following method was used. The 100 µg/ml thyroxine solution was diluted with ethanol to give tubes containg 0, 50, 100 and 500 ng thyroxine per tube. After evaporating off the ethanol solution under compressed air

at 40°C, 5 ml of human plasma was added to each tube. The tubes were left to equilibrate in a water bath at 30°C for 20 minutes after mixing on a vortex mixer. From each tube containing added cold thyroxine 200 µl plasma samples were aliquoted and extracted in duplicate with 2 ml of ethanol, vortexed for 15 seconds and then centrifuged for 10 minutes at 2500 rpm. The ethanolic supernatants were decanted into assay tubes and the assay procedure was continued as described above. The recovery was determined by plotting the results using a least squares regression analysis and found to be 77.2 %.

The intra assay coefficient of variation was determined using 14 determinations of a sample of human plasma and found to be 11.7 %.

Efficiency of thyroxine extraction from guinea pig plasma

Samples were prepared by adding ^{125} I labelled thyroxine to 100µl aliquots of plasma from ten different guinea pigs and extracting with 2 ml of ethanol.

The extraction efficiency was found to be 81.87±0.54% (Mean ± SEM: n=10)

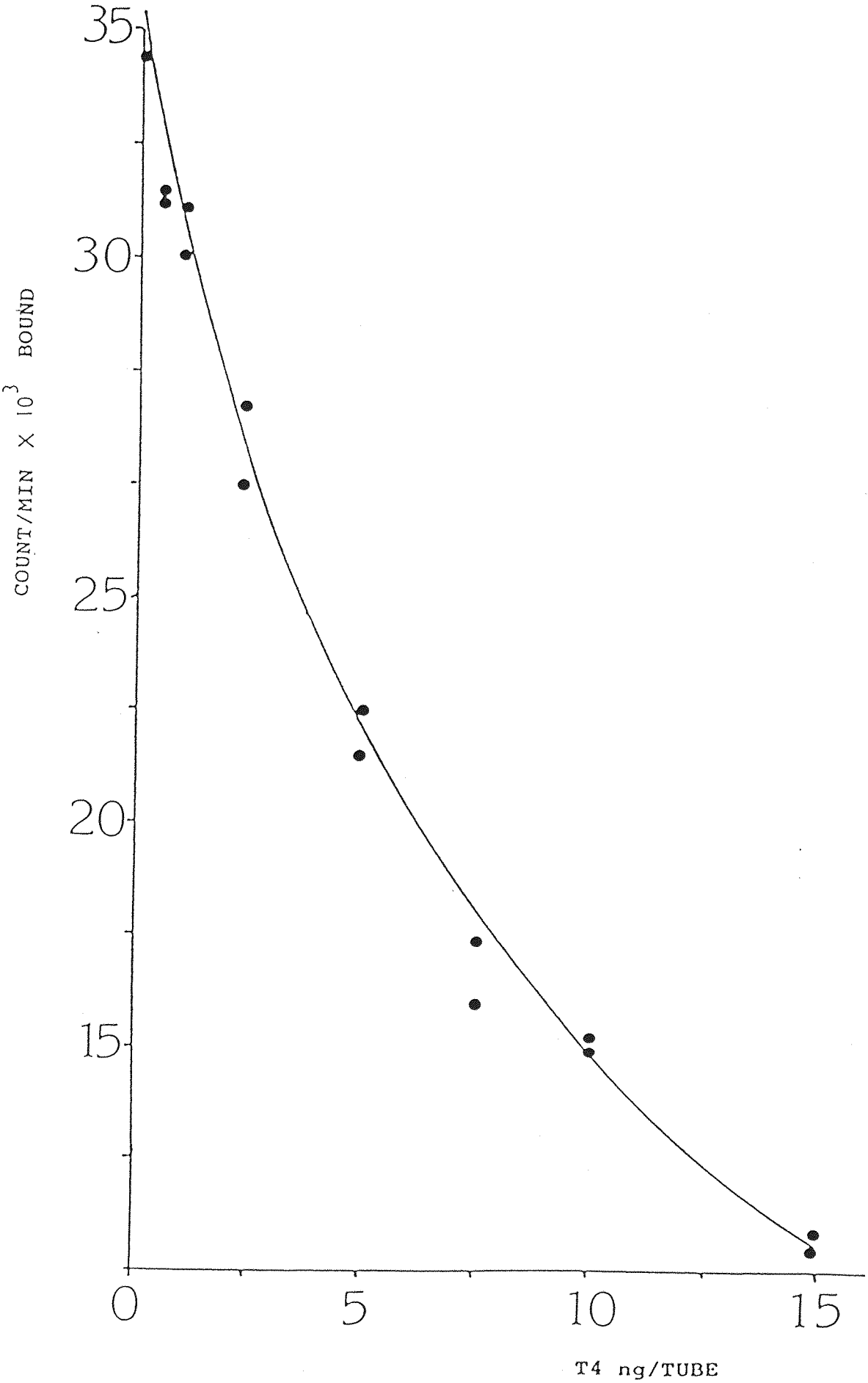


Figure M-9 A standard curve for the Thyroxine assay.

II. Triiodothyronine

Buffer Systems

Borate buffer (0.1 M, pH 8.6) was prepared by dissolving 8.25 g boric acid and 2.7 g sodium hydroxide in 500 ml distilled water and 3 ml of concentrated hydrochloric acid was then added carefully before the solution was diluted to 1 litre and then stored at 4°C for one week.

2. T3 labelled with ^{125}I to a high specific activity was obtained from RIA U.K. Ltd

3. A pre-precipitated T3 antibody complex was also obtained from RIA U.K. Ltd.

4. Standard T3 and rT3 solutions were prepared by dissolving 10 mg of T3 or rT3 (Sigma) in 100 ml of ethanol and stored in a plastic bottle at 4°C.

Method

Standards were prepared by diluting the 100 µg/ml T3 solution in ethanol to produce a series of tubes containing 0.01, 0.02, 0.05, 0.1, 0.25, 0.75, 1 and 2 ng of T3 (in duplicate) in 100 µl of ethanol. The same volume of ethanol was added to a further two tubes to act as a zero standard. Samples of plasma (50 µl) were aliquoted into LP3 tubes and 1ml of ethanol was added. The tubes were mixed on a vortex mixer and then centrifuged at 40°C and 2500 rpm for 10 minutes. The ethanolic supernatants were transferred into assay tubes.

The standard solutions and ethanolic extracts of plasma samples were evaporated to dryness under compressed air at 40°C. T3 complex, 500 µl, was added to all tubes and mixed before incubating in a 37°C water bath for 30 minutes. 200 µl of ^{125}I-T3 was added to

all tubes which were then vortexed before being put back into the water bath for a further 30 minutes. After this second incubation the tubes were centrifuged for 30 minutes at 4000 rpm. The supernatants were decanted and the precipitates were counted in a 1272 Clinigamma counter (LKB). Fig. M 10 shows a typical standard curve.

Recovery

The recovery of the assay was estimated by extracting and assaying guinea pig plasma containg added cold T3. The following method was employed. The 100 µg/ml T3 solution was diluted with ethanol and aliquoted to give tubes containing 5 ng T3 per tube. After evaporation of the ethanolic solution under compressed air at 40 °C one ml of guinea pig plasma was added to each tube. The tubes were left to equilibrate. After mixing on a vortex mixer at room temperature for 20 minutes aliquots of 50 µl of plasma were extracted with 1 ml of ethanol and centrifuged for 10 minutes at 2500 rpm. The ethanolic supernatants were transferred into LP3 tubes and assayed. The recovery was 106.51 %.

rT3 cross reaction

In order to test for cross-reaction of the T3 antiserum with rT3 standard rT3 solutions were prepared and diluted as above for T3. After the standard solutions were evaporated to dryness 500 µl T3 complex and 200 µl of ^{125}I T3 were added as in the normal T3 assay. A normal T3 standard curve was obtained but there was no displacement of the ^{125}I T3 by the rT3 standards indicating that the antibody does not cross react with rT3.

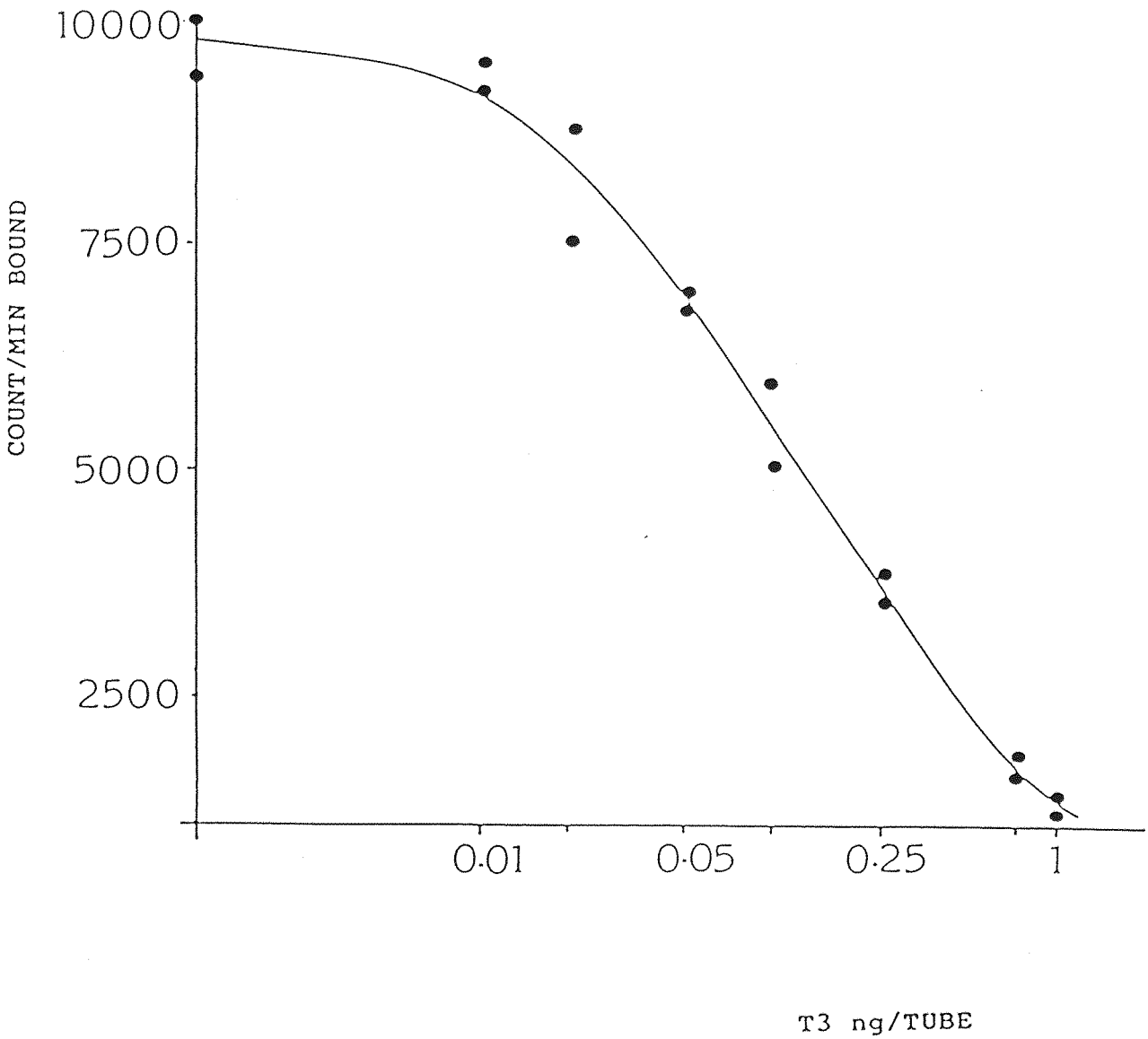


Figure M-10 A standard curve for the T3 assay.

III Free Thyroxine

The method used was similar to that described by Nathanielsz et al, (1973b). Except where stated otherwise all chemicals were obtained from BDH Chemicals Ltd, Poole, England.

Stock Solutions

All solutions were made up in distilled water and stored at 4°C .

1. Phosphate buffer (pH 7.4 0.15 molar) was prepared by dissolving 0.03 moles NaH_2PO_4 (4.68 g) and 0.12 moles Na_2HPO_4 (17 g) in 1 litre of distilled water.

2. Precipitating solution consisted of 10% $MgCl_2.6H_2O$ in tris sodium chloride buffer pH 9.3 (6.05 g tris, 5.9 g NaCl, 40 g NaI and 100 g $MgCl_2.6H_2O$ made up to 1 litre with distilled water).

3. Wash solution was made up as required in the same way as the precipitating solution except that the wash solution was titrated to pH 8.8 with hydrochloric acid.

4. Carrier thyroxine solution contained 250 mg T4 (L-thyroxine sodium salt, Sigma) 300 mg NaI dissolved in 250 ml of 0.05 M NaOH and stored in a plastic bottle since thyroxine adsorbs to glass.

5. Labelled thyroxine solution was prepared by diluting 30 micro curies of ^{125}I labelled T4 (Amersham plc) to 4 ml with phosphate buffer.

Method

Plasma samples of 0.2 ml were diluted with 4.7 ml of phosphate buffer and 0.1 ml of labelled T4 solution was then added.

The 5ml sample of diluted plasma was then pipetted into one side of a dialysis cell and 5 ml of phosphate buffer was added to the opposite side of the cell. Dialysis was carried out overnight in a water bath at 37°C. Equilibration is complete in this system within 16 hours. The concentration of ^{125}IT4 in the solutions on both sides of the cell was then measured. Fig. M 11 shows the arrangement of the cells and the means of access to the two compartments.

Aliquots of 2 ml were taken from each side of the cells and placed in counting tubes. Carrier solution (0.5 ml) was added to all tubes and they were vortexed briefly. Immediately after this 1 ml of precipitating solution was added. A dense white precipitate formed. The tubes were again vortexed and then centrifuged. The supernatant was decanted and discarded. Wash solution (2 ml) was then added to the precipitates, the tubes were vortexed centrifuged and the supernatants discarded. This washing procedure was repeated. The radioactivity of the final precipitates was then counted in a Biogamma counter.

Calculation

The percentage free thyroxine was corrected for dilution of the original plasma sample (Oppenheimer, Squef, Surks and Haver, 1963).

$$\text{Free thyroxine} = \frac{\text{cpm dialysate}}{(\text{cpm serum} - \text{cpm dialysate})} * DF * 100$$

where cpm dialysate = counts on dialysate side of membrane
cpm serum = counts on serum side of membrane
DF = dilution factor (1/25; 0.2 ml to 5.0 ml)

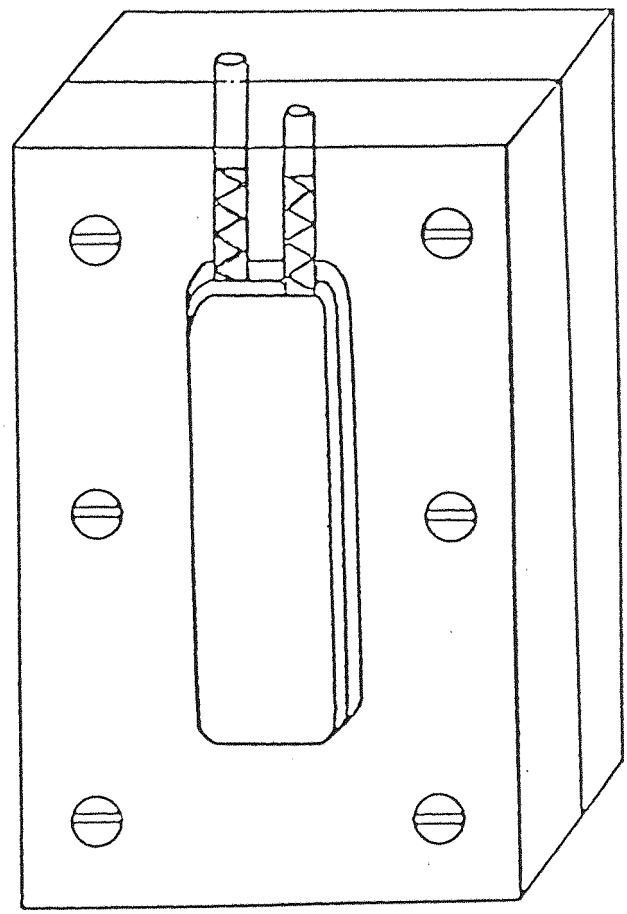


Figure M 11 Front view of an equilibrium dialysis cell to show the arrangement of the bolts and the means of access to the two compartments.

Statistics

Treated groups have been compared with normals as control. All result have been present as the mean ± the standard error of the mean, where n = the number of samples in each group. Significance limits have been determind using Student's test. All probabilities of 0.05 or less have been taken to be significant.

On the figure = mean ± SEM

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

**** = $P < 0.0001$

RESULTS

Results Section 1 - Growth

Introduction

There is little information regarding pre and post natal growth in the guinea pig. This first section describes the variations in whole body, thyroid, adrenal and pituitary gland weight during intrauterine life as well as in the young guinea pig.

Prenatal Growth

1. Growth of the body

A total of 219 fetuses were obtained from 55 pregnant guinea pigs ranging from 27 to 66 days of gestation. The average number of fetuses per pregnant guinea pig was three or four. The largest number found in a pregnant uterus was eight fetuses and the lowest one. The growth of a single fetus in one horn was influenced by the number (two or more) of fetuses in the other horn. A single fetus was found to weigh more than any fetus in the other horn.

Out of 123 fetuses whose location was recorded it was found that 58 were in the left horn and 65 in the right horn. The largest number in any horn was four. At the fifty five day stage the fetal sex can be determined by external examination. There were seventy four fetuses whose sex was determined. Of this number 37 were male and 37 female. In the right horn there were nineteen males and fourteen females while in the left horn eighteen males and twenty three females. The average weights of the thirty seven males was greater (59 $\pm$ 2.5 g) than the average weight of the thirty seven females (56 $\pm$ 2.65 g).

Figure R1:1 and Table R1:1 represent the mean weight of the fetuses at various gestational ages. There was a gradual increase in the body weight of the fetuses from 27 to 35 days. After that the weight increased linearly and more steeply until 62 days and then more rapidly again towards term. The curve plotted from the

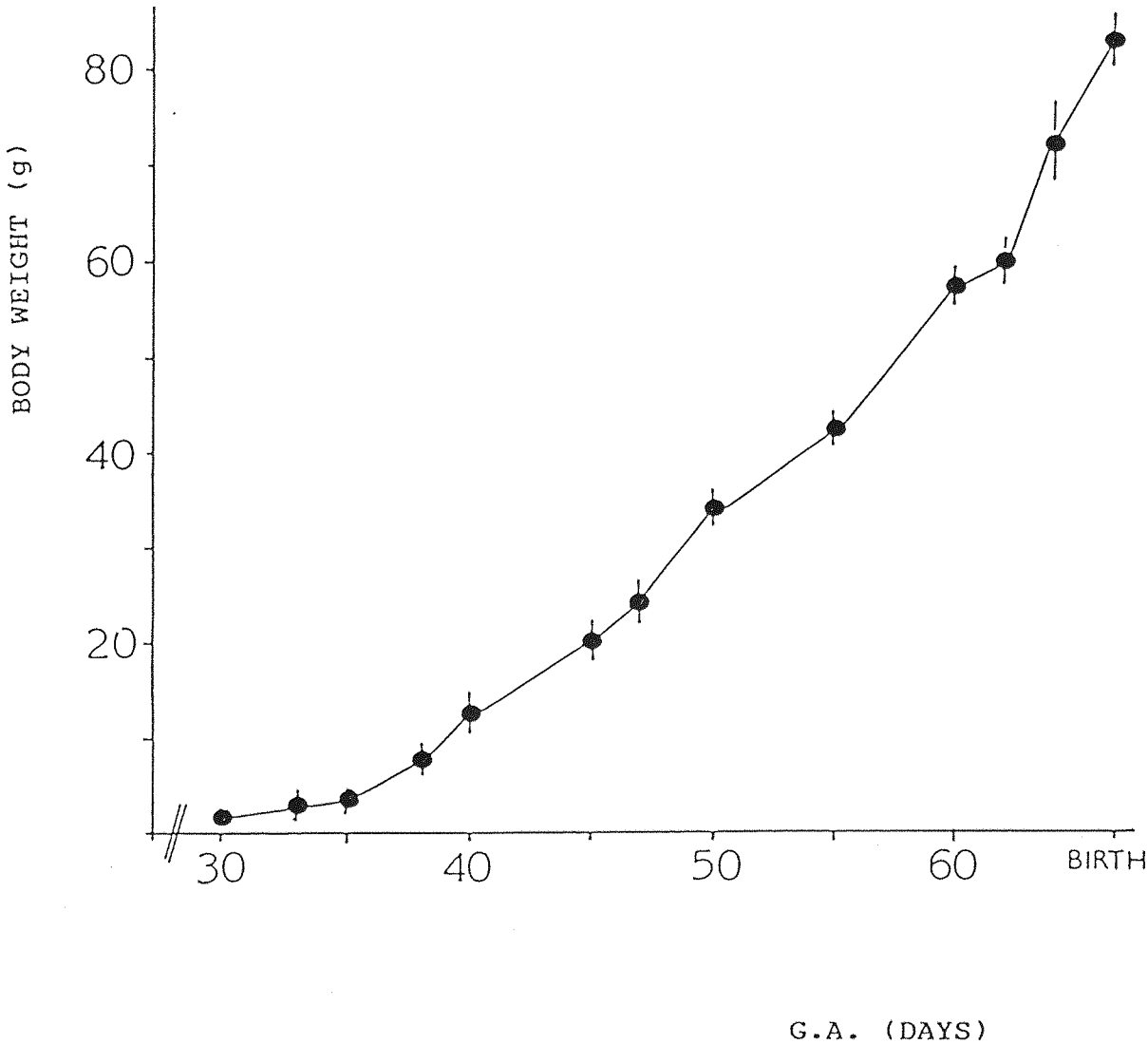


Figure R1-1 The mean body weight of guinea pig fetuses from 30 days gestational age to term.

Age in days	Body weight	Placental		Thyroid		Adrenal		Pituitary	
		g	%	mg	%	mg	mg%	mg	%
27 (3)	0.84 ± 0.02	0.48 ± 0.02	57.20 ± 1.44						
30 (6)	1.45 ± 0.10	0.71 ± 0.05	48.96 ± 6.63						
33 (7)	2.59 ± 0.48	0.76 ± 0.12	29.34 ± 2.17						
35 (10)	3.87 ± 0.18	1.20 ± 0.07	30.76 ± 0.90			1.23 ± 0.08	31.90 ± 1.54		
38 (4)	7.68 ± 0.10	1.78 ± 0.07	23.10 ± 0.68	3.53 ± 0.21	45.9 ± 2.32	2.41 ± 0.24	31.10 ± 3.02	0.94 ± 0.04	12.20 ± 0.51
40 (10)	11.9 ± 0.77	2.15 ± 0.10	18.06 ± 1.50	5.67 ± 0.43	48.2 ± 4.50	2.63 ± 0.20	22.10 ± 1.94	1.00 ± 0.00	8.40 ± 1.12
45 (12)	19.40 ± 1.01	3.23 ± 0.13	16.65 ± 0.55	8.67 ± 0.52	44.69 ± 2.53	3.35 ± 0.15	17.30 ± 1.04	1.51 ± 0.25	7.73 ± 0.72
47 (28)	24.30 ± 0.28	3.18 ± 0.14	12.90 ± 0.49	8.46 ± 0.24	35.50 ± 1.00	3.10 ± 0.16	12.80 ± 0.67	1.28 ± 0.10	5.26 ± 0.35
50 (10)	34.40 ± 0.95	3.44 ± 0.07	10.00 ± 0.33	13.8 ± 0.40	40.2 ± 1.11	7.60 ± 0.28	22.09 ± 0.97	1.63 ± 0.17	4.74 ± 0.66
55 (43)	42.90 ± 0.95	3.43 ± 0.17	8.08 ± 0.12	16.40 ± 0.51	38.60 ± 0.96	7.78 ± 0.40	18.13 ± 0.69	2.00 ± 0.14	4.66 ± 0.26
60 (26)	57.00 ± 0.93	4.20 ± 0.12	7.36 ± 0.41	21.20 ± 0.60	37.20 ± 1.16	9.85 ± 0.47	17.28 ± 0.90	2.42 ± 0.13	4.24 ± 0.25
62 (17)	64.00 ± 1.60	3.96 ± 0.13	6.18 ± 0.24	20.80 ± 0.40	32.80 ± 0.80	10.30 ± 0.30	16.20 ± 0.50	3.24 ± 0.12	5.06 ± 0.13
64 (15)	71.90 ± 2.70	4.00 ± 0.20	5.75 ± 0.21	23.19 ± 0.81	32.98 ± 0.57	10.80 ± 0.50	15.16 ± 0.80	3.18 ± 0.14	4.50 ± 0.36
65-67 (28)	83.30 ± 2.65	3.90 ± 0.34	5.21 ± 0.11	25.90 ± 1.32	30.94 ± 1.06	12.10 ± 0.64	14.50 ± 0.57	3.68 ± 0.20	4.42 ± 0.18

Table R1-1. Fetal body weight and placental weight (g). Absolute (mg) and relative (mg/100g body weight) weight of the fetal thyroid, adrenal and pituitary glands.

relation of weight to age represents the absolute increases in weight from stage to stage but not the rate of growth. The rate of growth may be calculated as the increase in weight per day over a period of days expressed as a percentage of the body weight at the beginning of the period. The curve of Figure R1:2 starts from a high rate of growth in the early stage of development followed by a decline to 24.7 % at 35 days, a rise to 32.55 % at 38 days and then there was a drop to 5 % at 55 days. The growth rate increased slightly at 60 days and then declined towards term.

Table R1:2 gives the weight according to the litter size for 219 fetuses and shows the average weight of the fetuses based on the number of days in utero and the number of individuals in each litter. Before 50 days the rate of fetal growth is approximately the same for individuals in the litters of all sizes, while from 50 days and later the growth rate of the fetuses depends largely upon the number of fetuses in the uterus and divergencies begin to appear amongst litters of different ages. The slower rate of growth was found in the largest litter size where in some cases fetuses weighed about half as much, or less, as the normal full term weight.

2. Growth of the placenta

The weight of the placenta ran closely with the fetal weight. Figure R1:3 and Table R1:3 show the relationship between the weight of the placenta to the number of days in the uterus. There was a linear increase in the placental absolute weight from 33 days to 45 days of gestation. From 45 to 55 days there was a very small increase in weight and from 55 days to 60 days there was a significant increase ($P<0.01$) in the placental weight. After 60 days, as shown by the figure, the absolute weight of the placenta does not increase. When placental weight was expressed per 100 g fetal body weight (Fig. R1-4) the placenta growth at early stages of pregnancy (27 and 30 days) was seen to be greater such that the weight of the placenta was approximately half the fetal body weight. After that the fetal weight increased rapidly and the placental growth rate

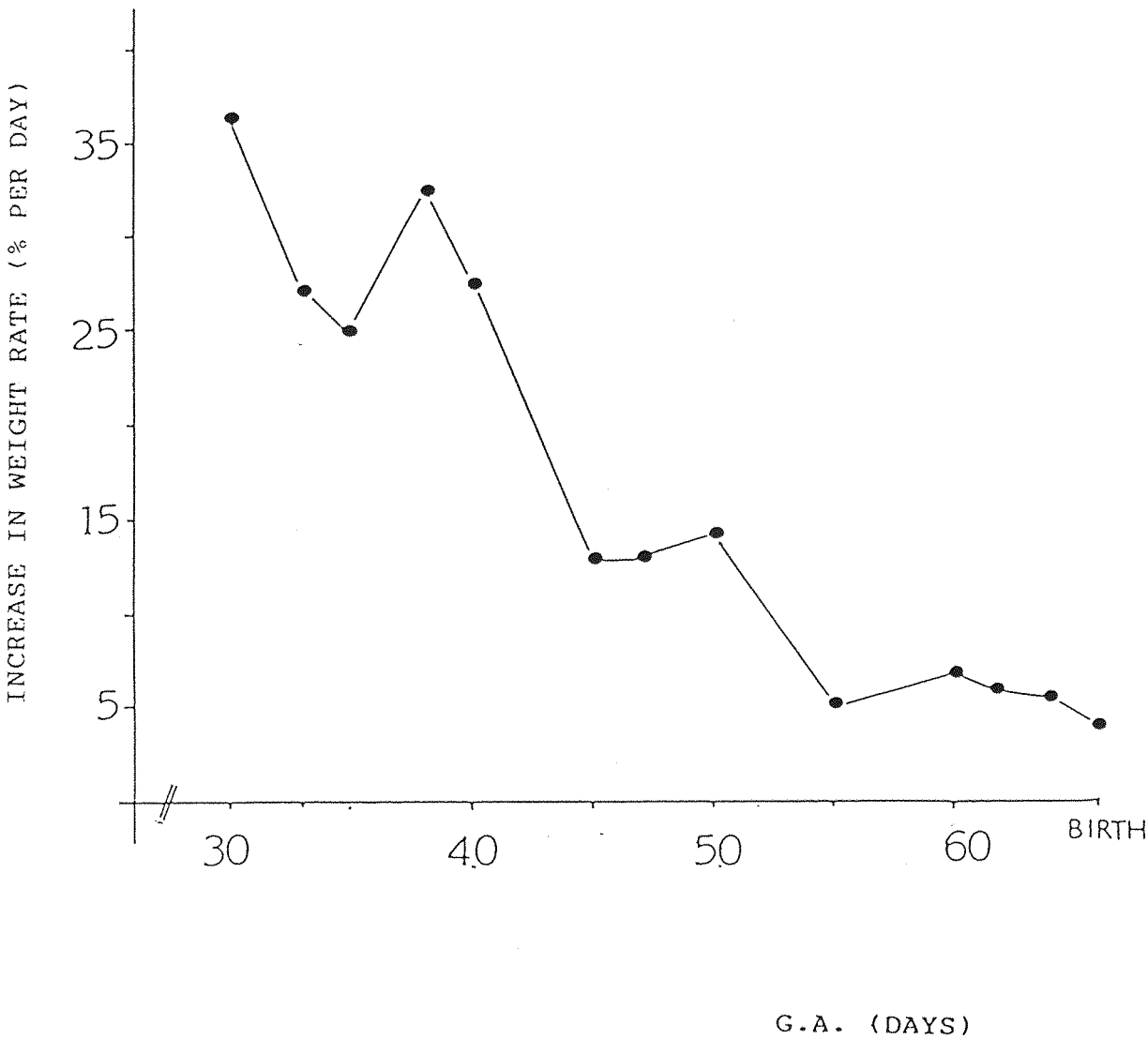


Figure R1-2 The rate of fetal growth expressed as the percentage increase in body weight per day from 30 days gestational age to term.

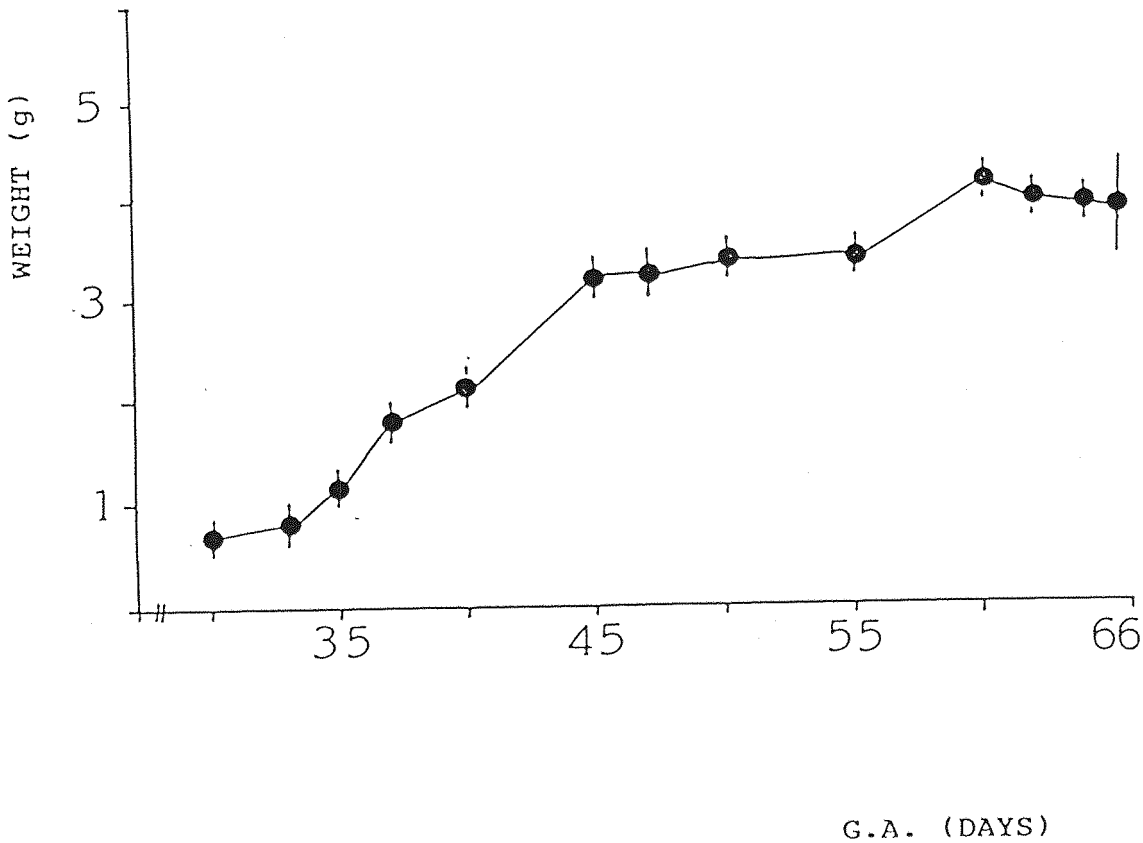


Figure R1-3 Mean placental weight from 30 days gestational age to term.

Gestational age in days	Number of fetuses in uterus						Averge for all litter size
	1	2	3	4	5	6	
27			0.84				0.84 ± 0.92
30		1.2		1.58			1.45 ± 0.10
33			1.39	3.51			2.59 ± 0.48
35			4.11	3.5			3.87 ± 0.18
38			7.68				7.68 ± 0.10
40			10.81	13.5			11.90 ± 0.77
45	22.3		23	17.66			19.40 ± 1.01
47			25	23.5	23.9	25.33	24.30 ± 0.28
50		39	34.8		32.2		34.40 ± 0.95
55			50.66	47.38	42.65	38.25	42.70 ± 0.95
60				57.06	57		57.00 ± 0.93
62				67.10	61.25		64.00 ± 1.60
64			82.17	63	66.8		71.90 ± 2.70
67		94.5	91.33	85.87		76	83.30 ± 2.65

Table R1-2. Average weight (g) of fetuses based on age and the number of fetuses in the uterus.

Days in utero	Number of placentae in uterus						Average for all litter sizes
	1	2	3	4	5	6	
27			0.48				0.48 ± 0.02
30		0.85		0.64			0.71 ± 0.05
33			0.5	0.96			0.76 ± 0.12
35			1.21	1.08			1.20 ± 0.07
38			1.78				1.78 ± 0.07
40						2.15	2.15 ± 0.10
45	3		3.3				3.23 ± 0.13
47				2.9	3.42		3.18 ± 0.14
50		3.45	3.43				3.44 ± 0.07
55					3.79	2.83	3.43 ± 0.17
60				4.2			4.20 ± 0.12
62				4.3	3.68		3.96 ± 0.13
64			4				4.00 ± 0.15
67				4.45		3.5	3.90 ± 0.34

Table R1-3. Average weight (g) of placentae based on the age and the number of placentae in the uterus.

declined steadily with increasing age. At 50 days the relative placental weight percentage was less than 10 % of fetal body weight and between 62 days and birth the relative placental weight reached 5 %. The average weight of the placentae based on the number of placentae in the uterus and gestational age is given in Table R1-3. From 27 days to 50 days the growth rate of the placenta was approximately the same for all litters. From 55 days to full term the placental weight was influenced by the crowding in the uterus and it seems that as the number of individuals in the litter increases mean placental, like mean fetal, weight decreases while the total placenta weight of the litter increases. Individual placental weight was greater in small than in large litters.

3. Changes in thyroid weight

The growth of the thyroid gland in relation to body weight in the fetal guinea pig from 38 days of pregnancy to term was recorded. From Figure R1:5 and Table R1:1 it may be seen that there was a linear increase in the absolute weight of the thyroid gland from 38 days of intra-uterine development towards birth, regardless of individal variations in the body weights at each age. Thyroid weight in fetal guinea pigs is nearly directly proportional to body weight. Figure R1:4 and Table R1:1 show there was variation in growth rate of the fetal thyroid gland when expressed per 100 g fetal body weight. Relative thyroid weight was increased during the period of 38 to 40 days and also in the period of 47 to 50 days gestation. There was a decline in the relative weight from 40 days to 47 days and from 50 days towards term.

4. Changes in adrenal weight

The increase in adrenal weight with respect to body weight is illustrated in Figure R1:5 and Table R1:1 The adrenal weight of the fetus showed a continuous upward trend. From 35 to 47 days there was a gradual increase in adrenal

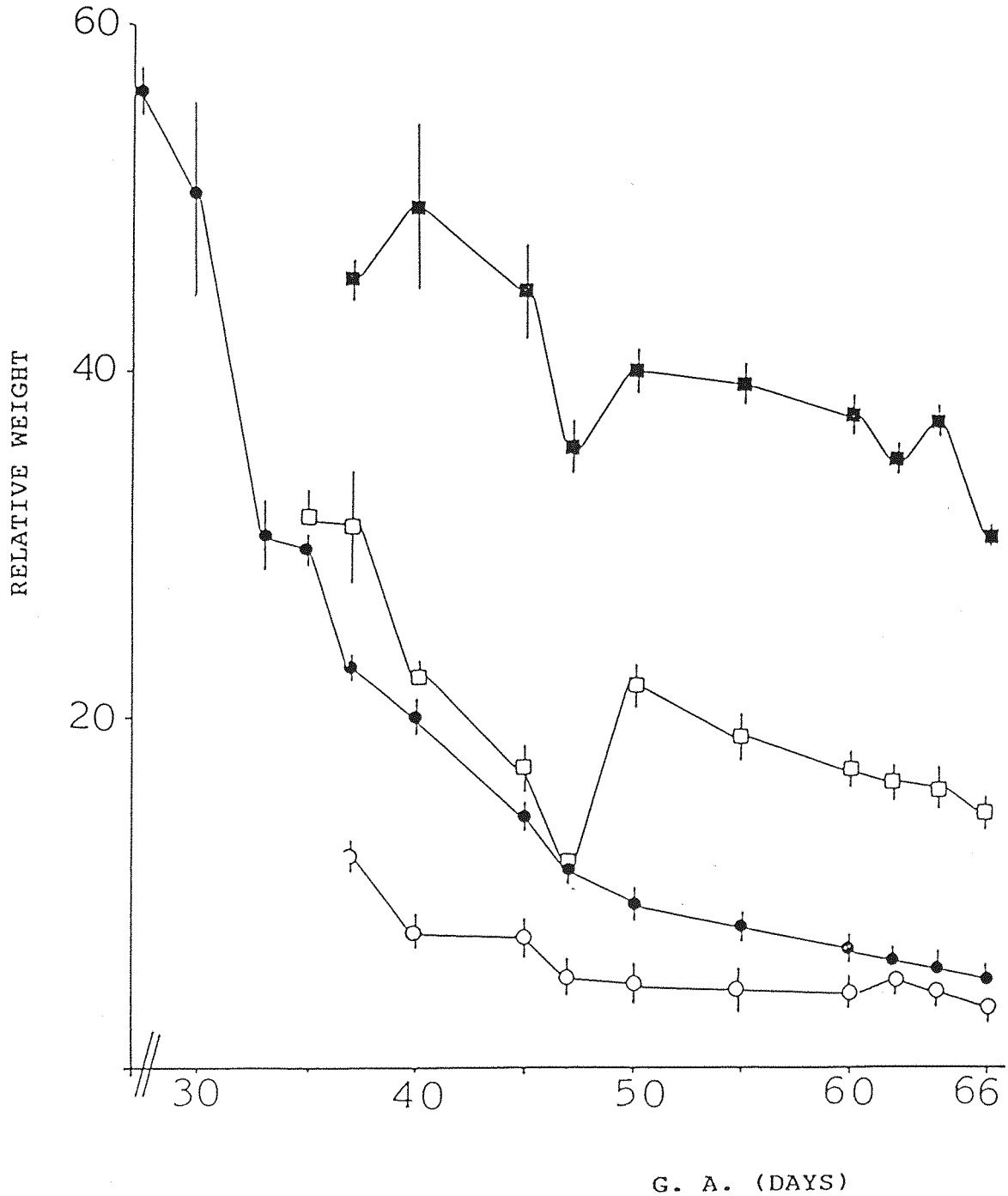


Figure R1-4 The relative placental weight (g/100g fetal body weight)(●) and relative fetal thyroid (■), adrenal (□) and pituitary weights (○) in (mg/100g fetal body weight). From 27 days gestational age to term.

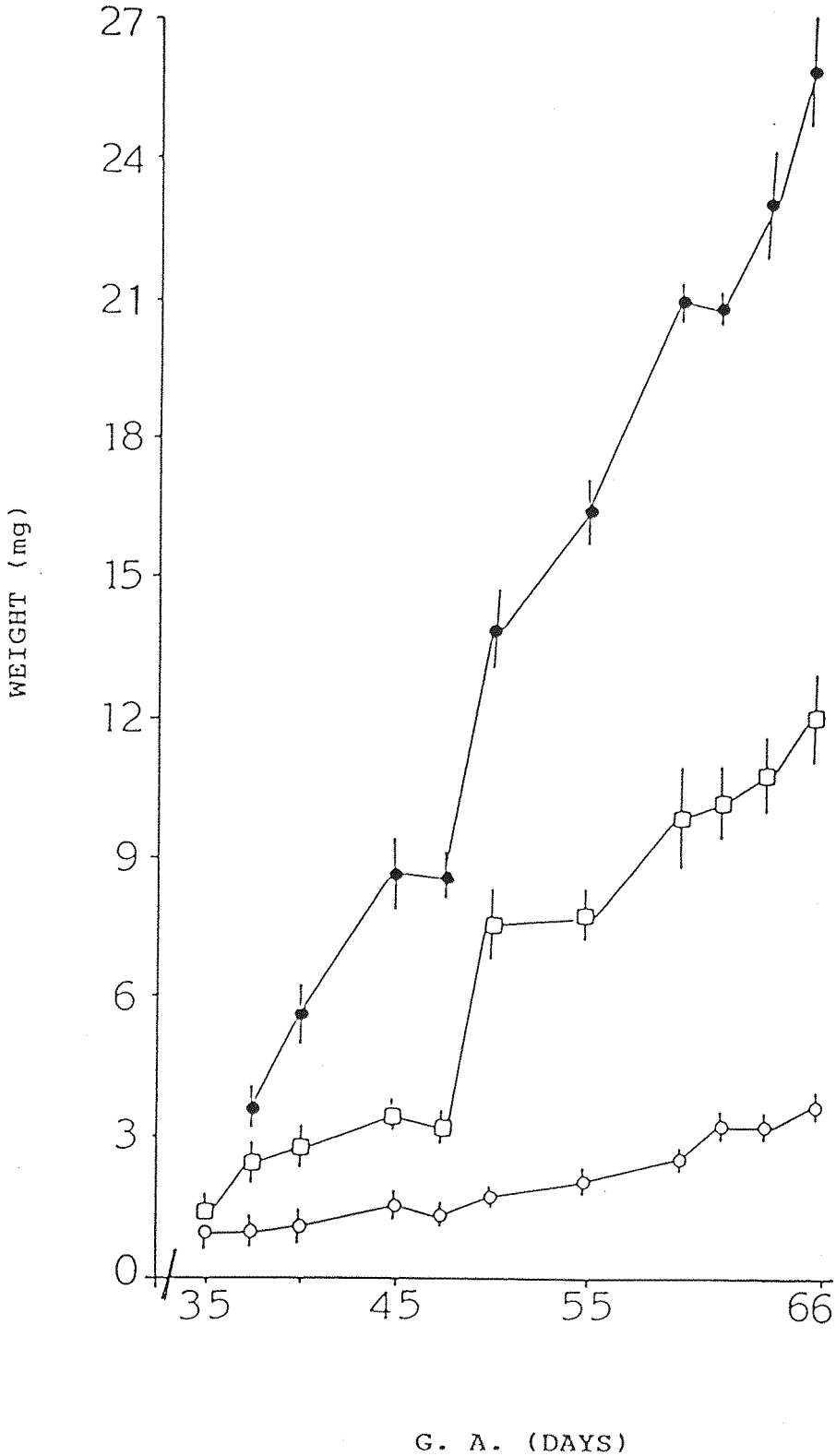


Figure R1-5 The absolute weight (mg) of the thyroid (●), adrenal (□), and pituitary (○) glands of guinea pig fetuses from 35 days gestational age to term.

absolute weight but from 47 to 50 days an increase occurred ($P < 0.001$). From 55 to 60 days there was also a significant increase in adrenal weight ($P < 0.001$). After 60 days there was a gradual increase in weight towards birth. Figure R1:4 and Table R1:1 show that the growth of the adrenal gland compared to body growth declined sharply from a relative weight of 32 mg/100g at 35 days to 13 mg/100g at 47 days and then increased to 22.2 mg/100g at 50 days and declined gradually towards term. Thus there seems to be a surge of adrenal growth at about 47 to 50 days of gestation.

5. Changes in pituitary weight

In early stages of pregnancy the pituitary gland of fetuses showed a slow increase in absolute weight. Pituitary average weight was increased from 1.6 mg at 50 days to 2.21 mg at 55 days of intra-uterine life and from 2.42 mg at 60 days to 3.29 mg at the 62 days stage. Figure R1:5 shows there was a more rapid increase in the pituitary weight from 60 days towards full term. The ratio of pituitary weight to 100 gm body weight was calculated. At 37 days the relative weight of the pituitary gland was 12.2mg/100g. This declined to 7.74 mg/100g at 40 days and to 5 mg/100g at 47 days after that there was a gradual decrease in the relative weight towards full term (Figure R1:4 and Table R1:1)

Postnatal growth

1. Body weight

The growth of the newborn guinea pigs was studied in 97 young animals obtained from 26 litters followed from the first day of postnatal life until weaning. Data describing the studies are given in table R1:4 . It was found that the litters with the smallest number of individuals had the highest individual birth weight (112g) whilst the largest litters (7 fetuses) had the lowest mean birth weight (64g). There was a significant increase ($P<0.001$) in the average body weight by the 2nd, 5th, 6th, 8th and 8th day of postnatal life in the young born from litters of 1,2,3,4,and 5 fetuses respectively (Table R1:4). However a significant increase ($P<0.05$) in body weight of those young born in litters of 7 individuals did not occur until the 8th day of postnatal life. In litters of one or two fetuses the males and females were born with approximately the same body weight and the body weights for both sexes ran closely together until weaning (Fig.R1:6-a & R1:6-b). The males from litters of 3 fetuses (Fig. R1:6-c) were born heavier (105g) than the females (91g) this difference decreased with age. At 11 days of age the mean body weight of both sexes was 148g and the weights then remained similar until weaning. The mean body weight of the males born to litters of 4 individuals was found to be more than that of the females from birth to weaning (Fig. R1:6-d). Figure R1:6-e illustrates the growth of young born to litters of 5 fetuses. The mean body weights of the sexes were similar at early stages of life and divergencies appeared at increasing age. The mean body weight of males and females born to litters of 7 fetuses increased slowly and ran closely from birth until weaning (Fig. R1:6-f).

Figure R1:7 and R1:8 show the increase in mean body weights of male and female young born to different litters. Males and females born to small litters of 1 or 2 individuals were found to weigh considerably more than those born to litters of 7 individuals. This significant difference ($P<0.001$) was maintained down to litters of 5 individuals in the case of the males and 4 individuals in the

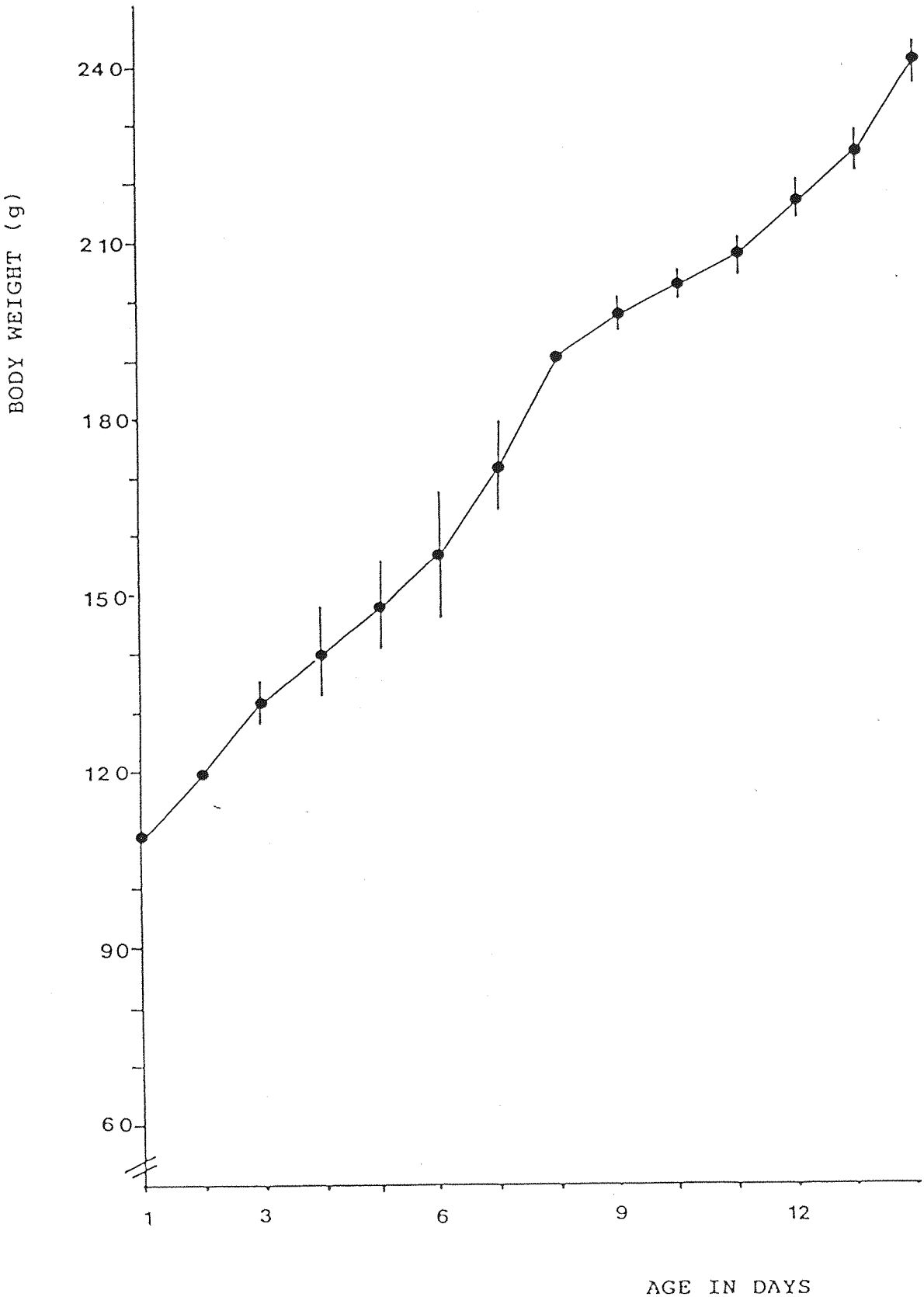


Figure R1-6a The mean body weight of female (●) guinea pigs from litters of 1 fetus from 1-14 days post-partum.

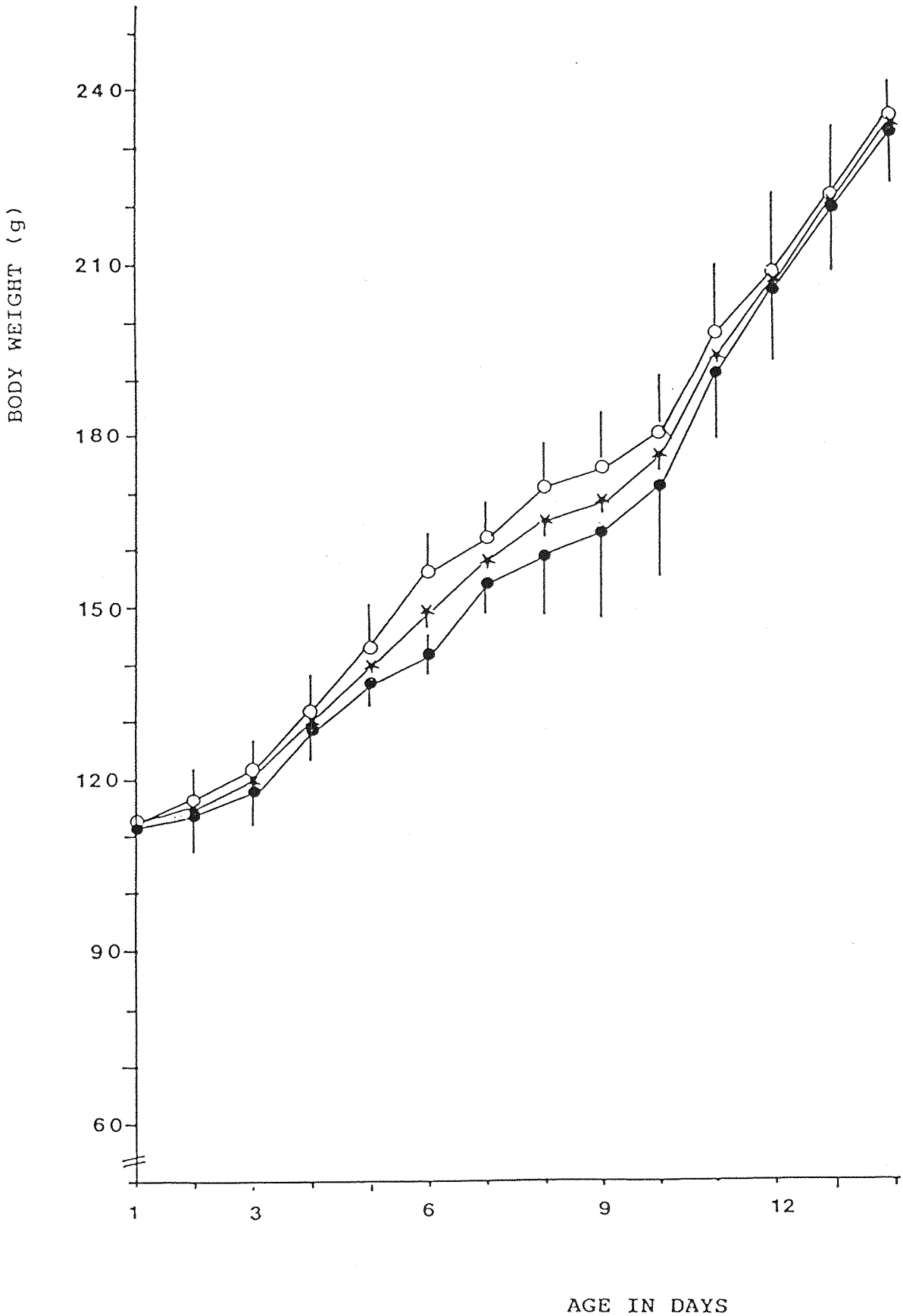


Figure R1-6b The mean body weight of male (●), female (○) and all guinea pigs (★) from litters of 2 fetuses from 1-14 days post-partum.

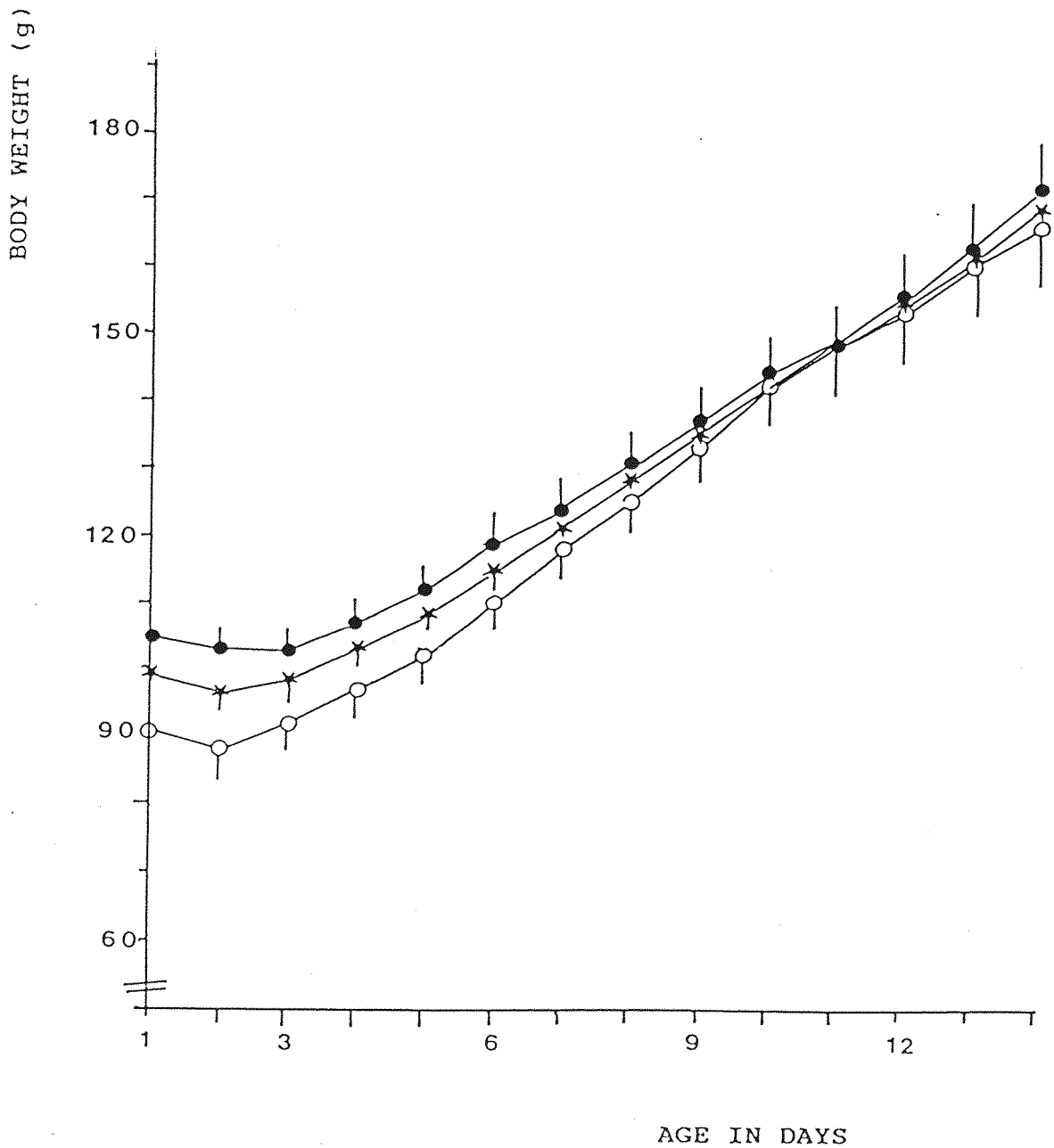


Figure R1-6c The mean body weight of male (●), female (○) and all guinea pigs (★) from litters of 3 fetuses from 1-14 days post-partum.

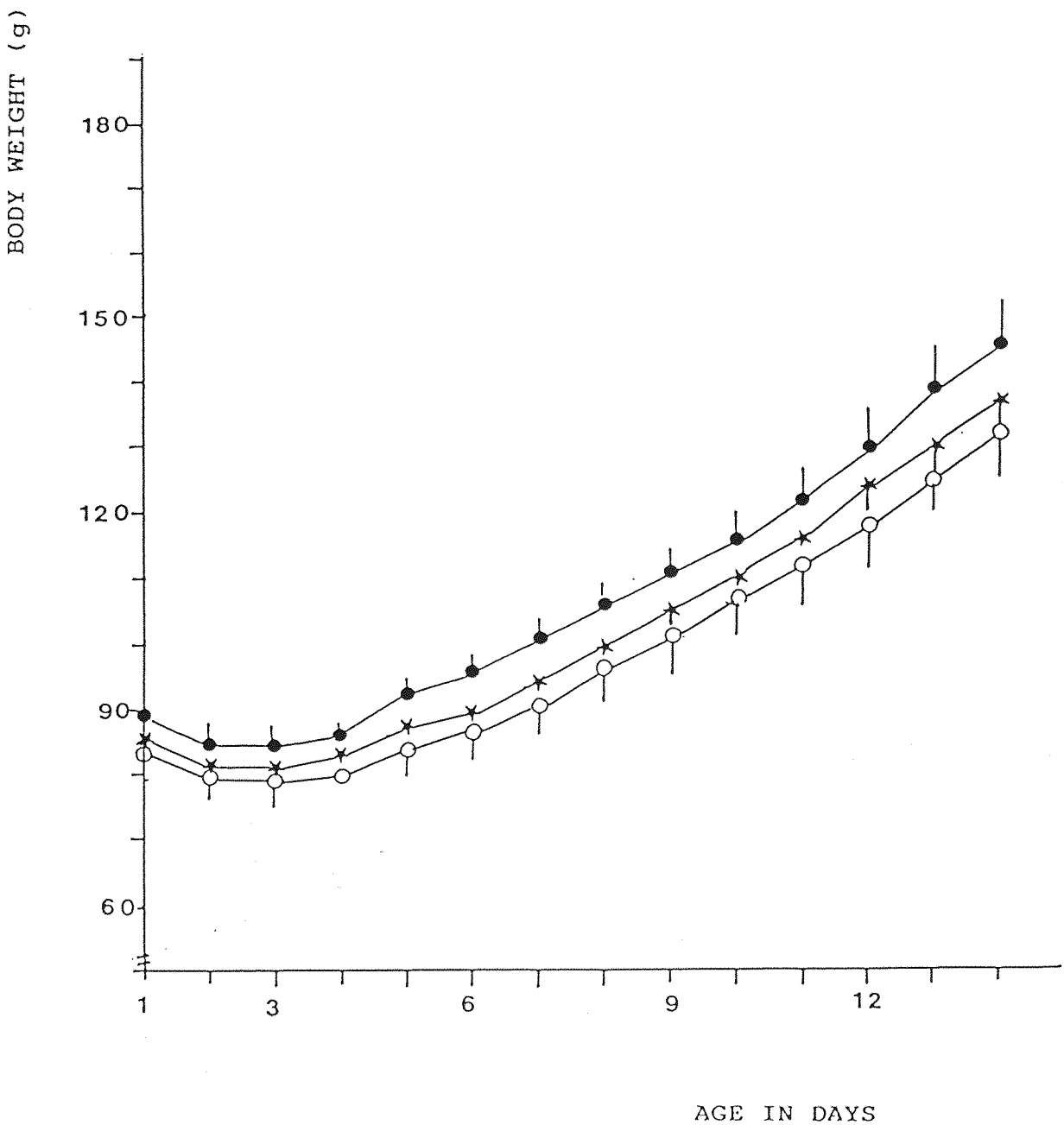


Figure R1-6d The mean body weight of male (●), female (○) and all guinea pigs (★) from litters of 4 fetuses from 1-14 days post-partum.

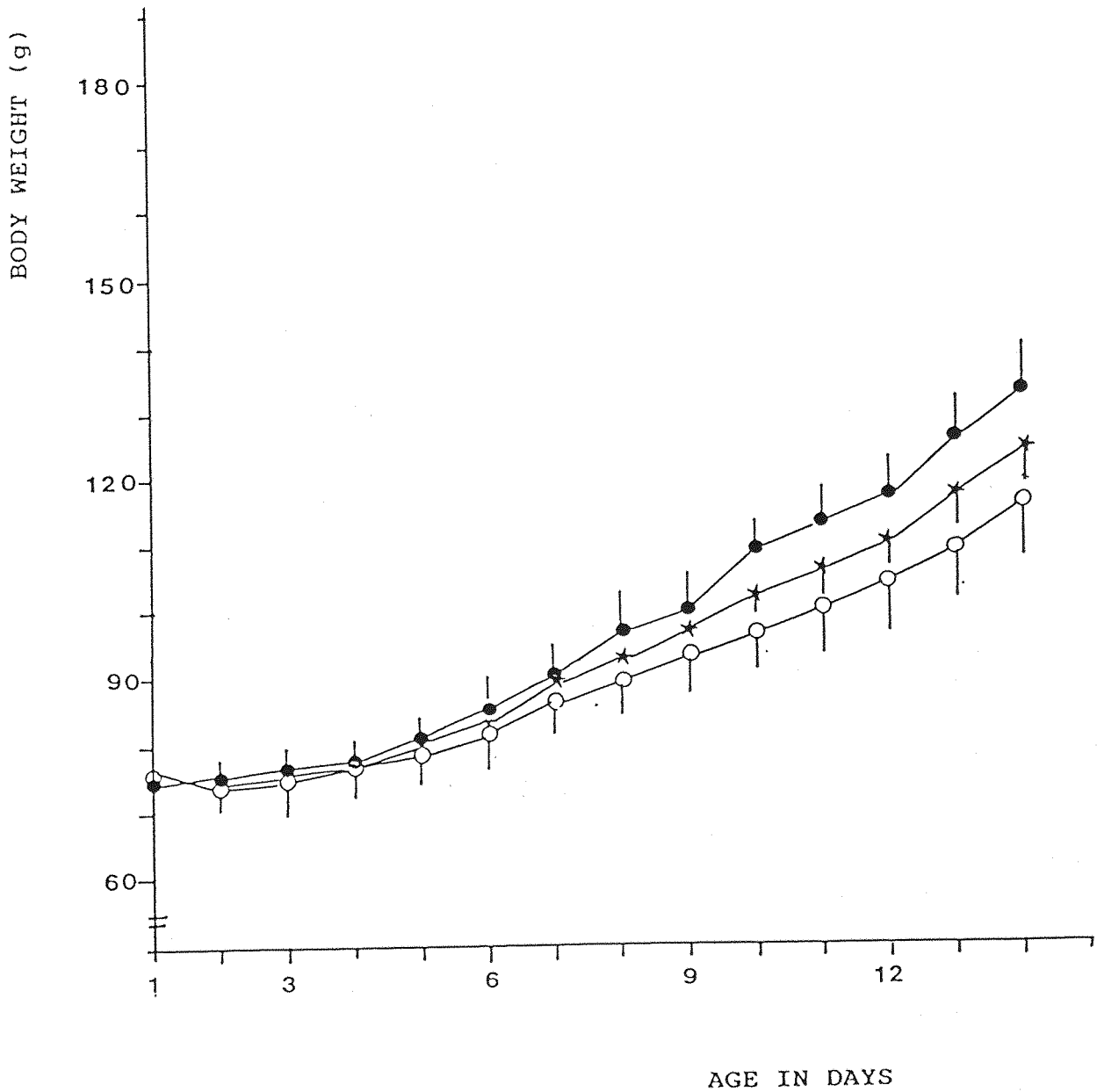


Figure R1-6e The mean body weight of male (●), female (○) and all guinea pigs (★) from litters of 5 fetuses from 1-14 days post-partum.

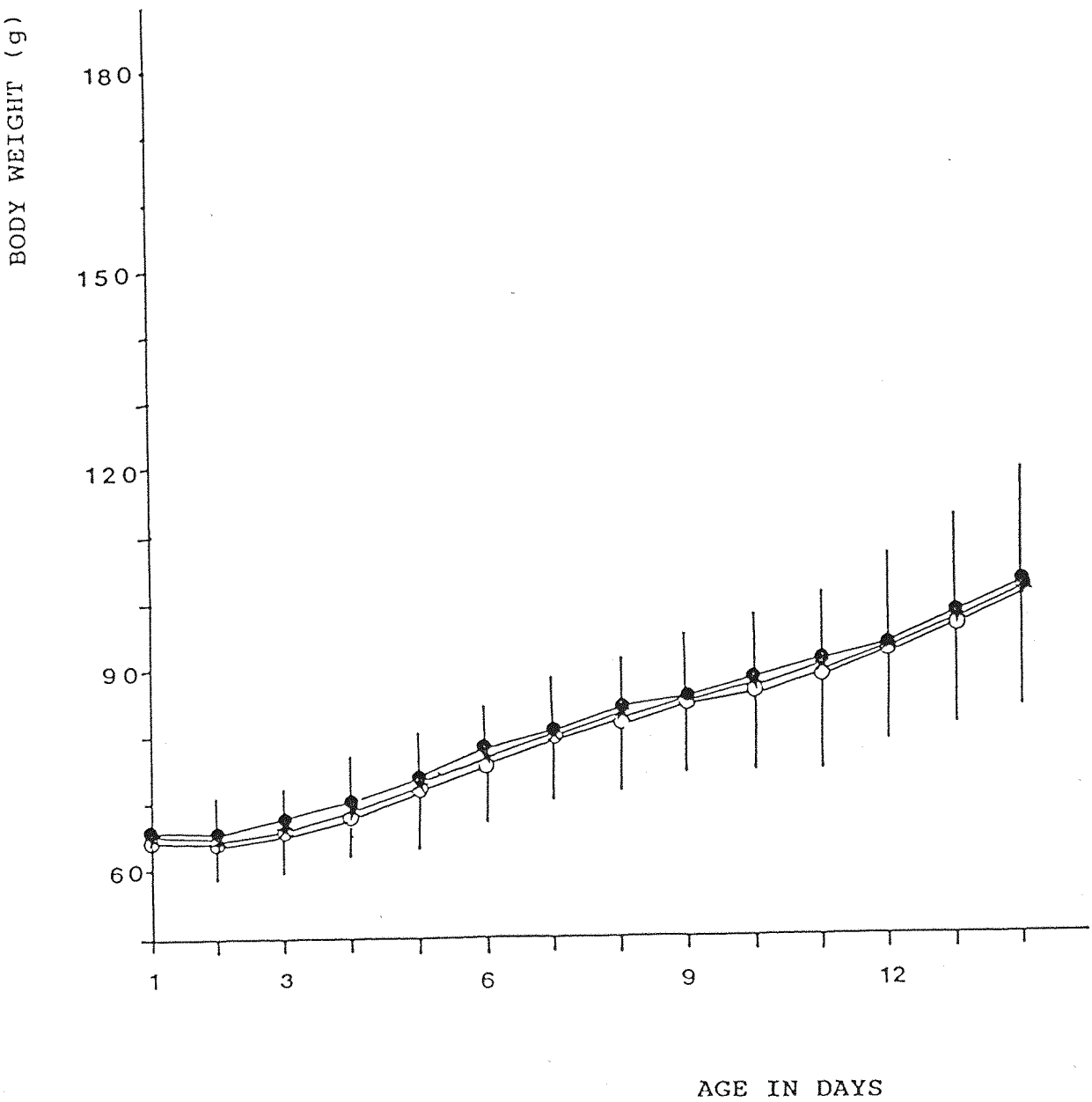


Figure R1-6f The mean body weight of male (●), female (○) and all guinea pigs (★) from litters of 7 fetuses from 1-14 days post-partum.

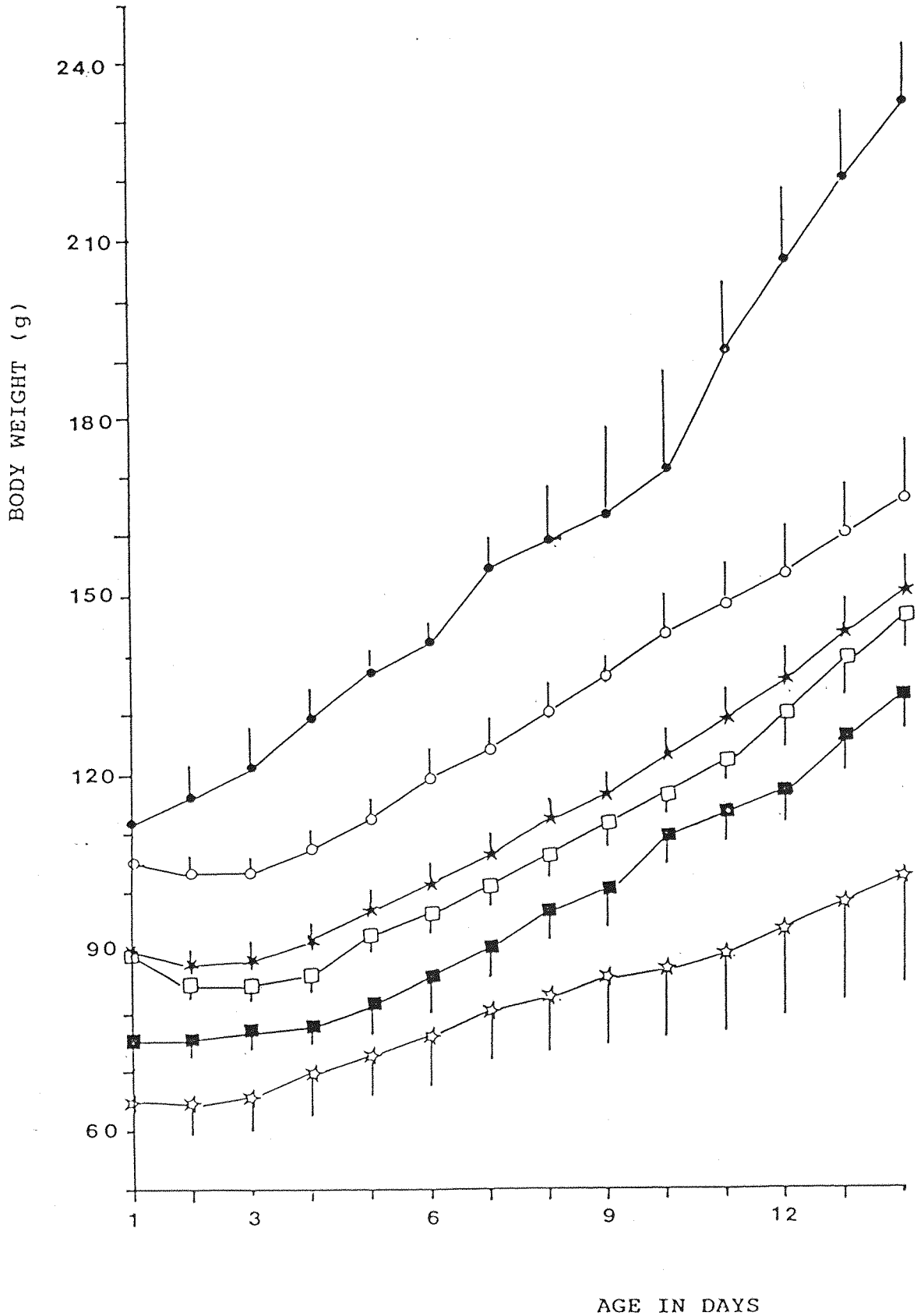
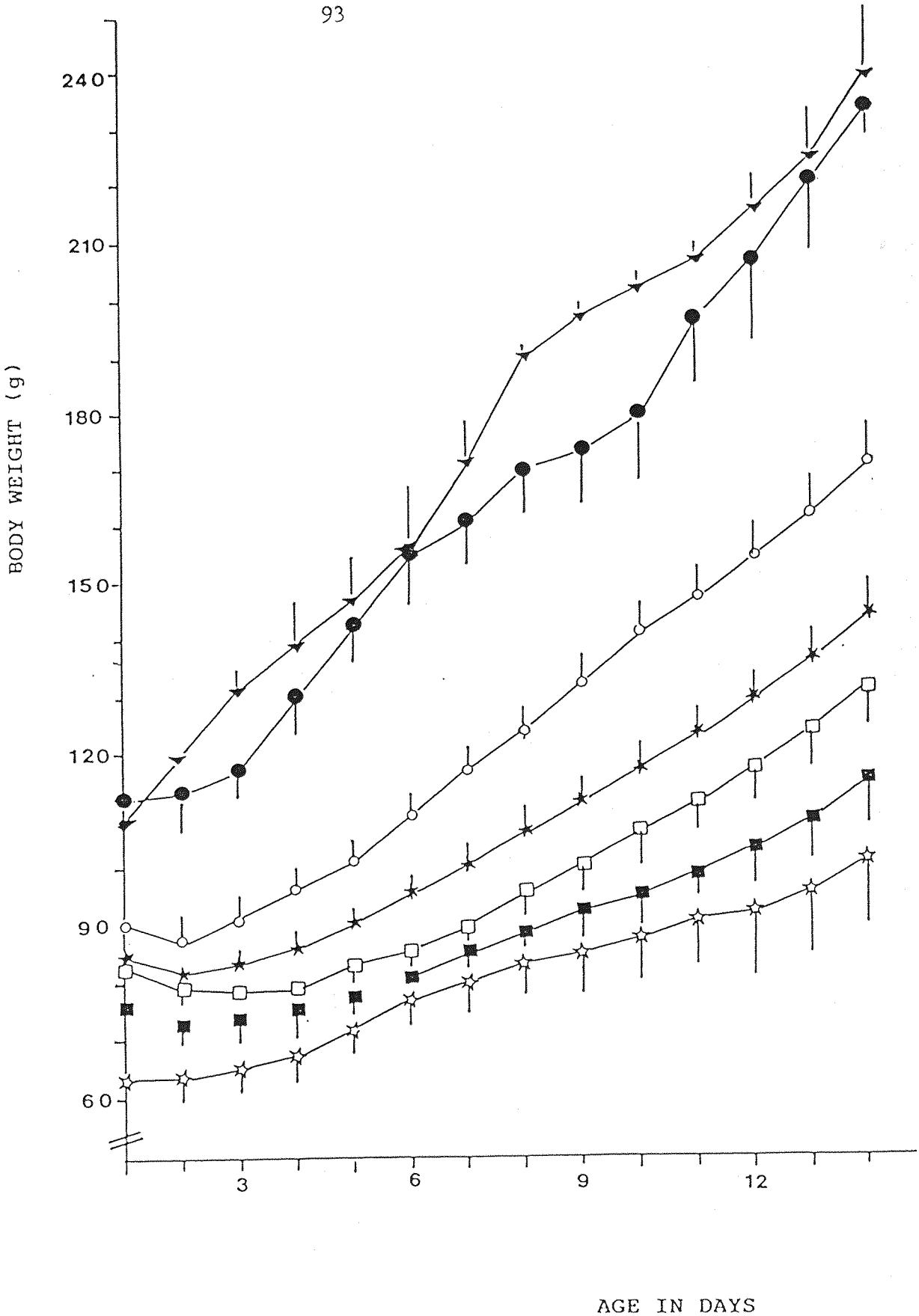


Figure R1-7 The increase in mean body weight of male guinea pigs from litters of different sizes. Litters of 2 (●), 3 (○), 4 (□), 5 (■) and 7 (☆) pups and all groups combined (★).



Fihure R1-8 The increase in mean body weight of female guinea pigs from litters of different sizes. Litters of 1 (▼), 2 (●), 3 (○), 4 (□), 5 (■) and 7 (☆) pups and mean (★).

case of the females. It was found that the mean body weight of the males from litters of 2,3,4,5 and 7 fetuses showed an increase compared to birth weight by days 2,4,5,3 and 3 respectively. The mean body weights of the males were not significantly increased until days 5,8,8,9 and N.S. respectively (Figs R1-8). The mean body weight of the female young from litters of 1,2,3,4,5, and 7 individuals was found to be greater than birth weight by days 2,2,3,6,5 and 3 respectively but these increases were not significant until days 2,5,7,10,13 and N.S. respectively. The average body weights for males and females from all litters combined were increased by day 4 of postanatal life and this increase became significant ($P<0.001$) by day 7 (Fig. R1-9).

Figure R1-10 and Table R1-4 illustrate the mean body weights of the newborn guinea pigs from the different litters. It was found that the means of the body weights of guinea pigs from litters of one or two individuals started to increase from the second day of life whilst the average body weight of the young born to litters of 3,4,5 and 7 individuals began to increase at days 4,5,4 and 3 days respectively (Figure R1-10). The average weight of the young from all litters was found to be 86.9g on the day of birth. This weight was decreased on the 2^{nd} and 3^{rd} days of postanatal life had increased above birth weight by day 4 but not significantly ($P<0.001$) so until day 6. A summary of the absolute increases in pre and postnatal life is shown in Figure R1-11. A linear growth started at 33 days of gestation and accelerated towards birth. After birth there was a decline in average body weight until the sixth postnatal day after which weight continued to increase until weaning.

2. Changes in thyroid weight

From the data in Table R1:6 it may be seen that there was a decline in the absolute weight of the thyroid gland at day 2 but this was not significant ($P<0.05$) until days 3 and 4. Gland weight then increased and this increase continued as body weight increased (Fig. R1-12). The relative weight of the gland

expressed as mg/100g body weight declined steadily in the neonatal period from 34 mg/100g at day 1 to 17 mg/100g by day 21 (Fig. R1-13).

3. Changes in adrenal weight

The increase in adrenal weight with respect to age is shown in Figure R1-14 and Table R1-5. The adrenal weight increased from 11 mg at birth to 44.8 mg at day 21. The relative weight of the adrenal increased from 14 mg/100g at day 1 to 22 mg/100g at day 4 and then decreased to 20 mg/100g at day 6 and remained unchanged until weaning (Figure R1-15).

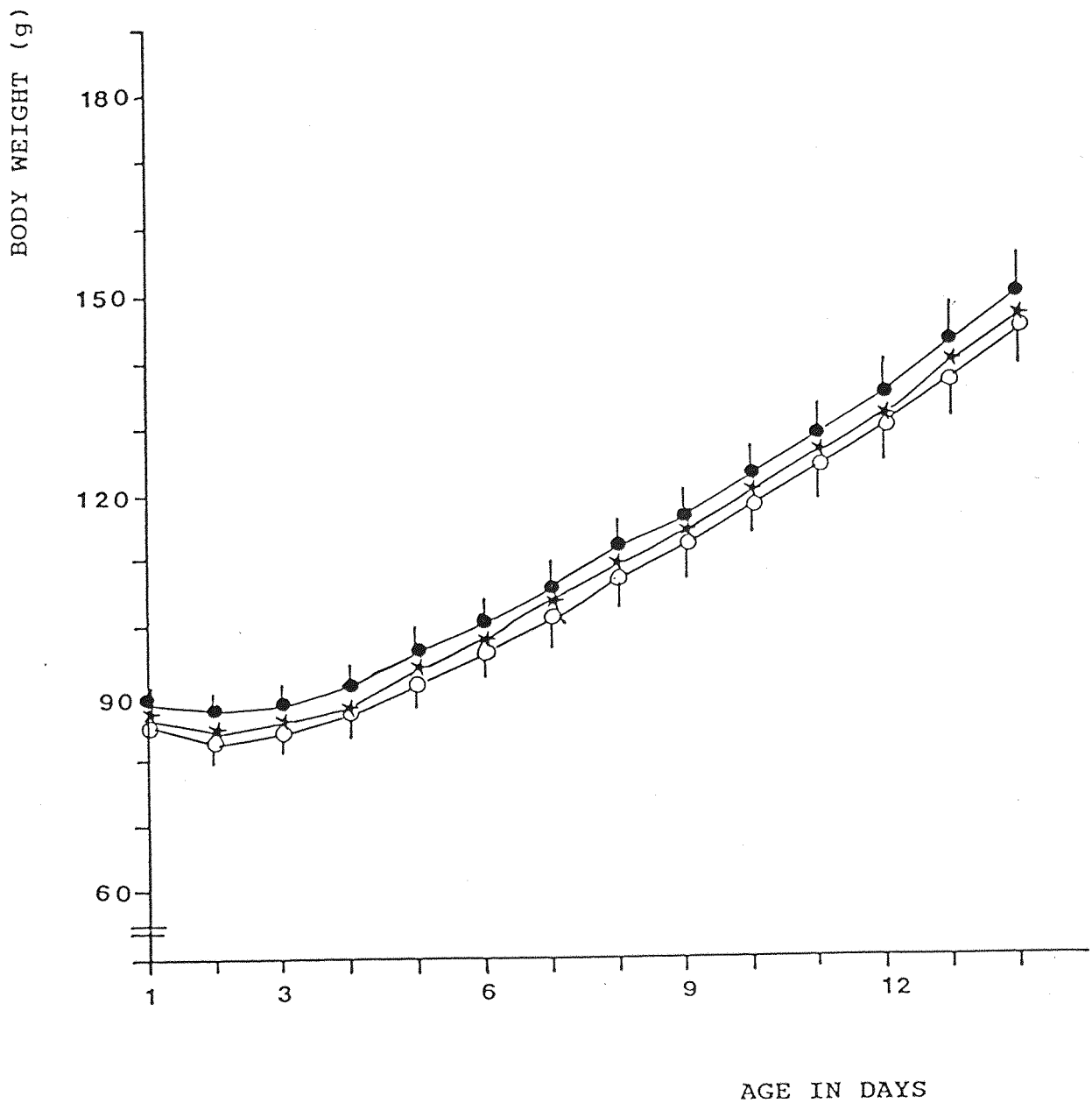


Figure R1-9 The average of the daily increase in body weight of neonatal guinea pigs from all litters. Females n=53 (○), males n=46 (●) and both combined n=99 (★).

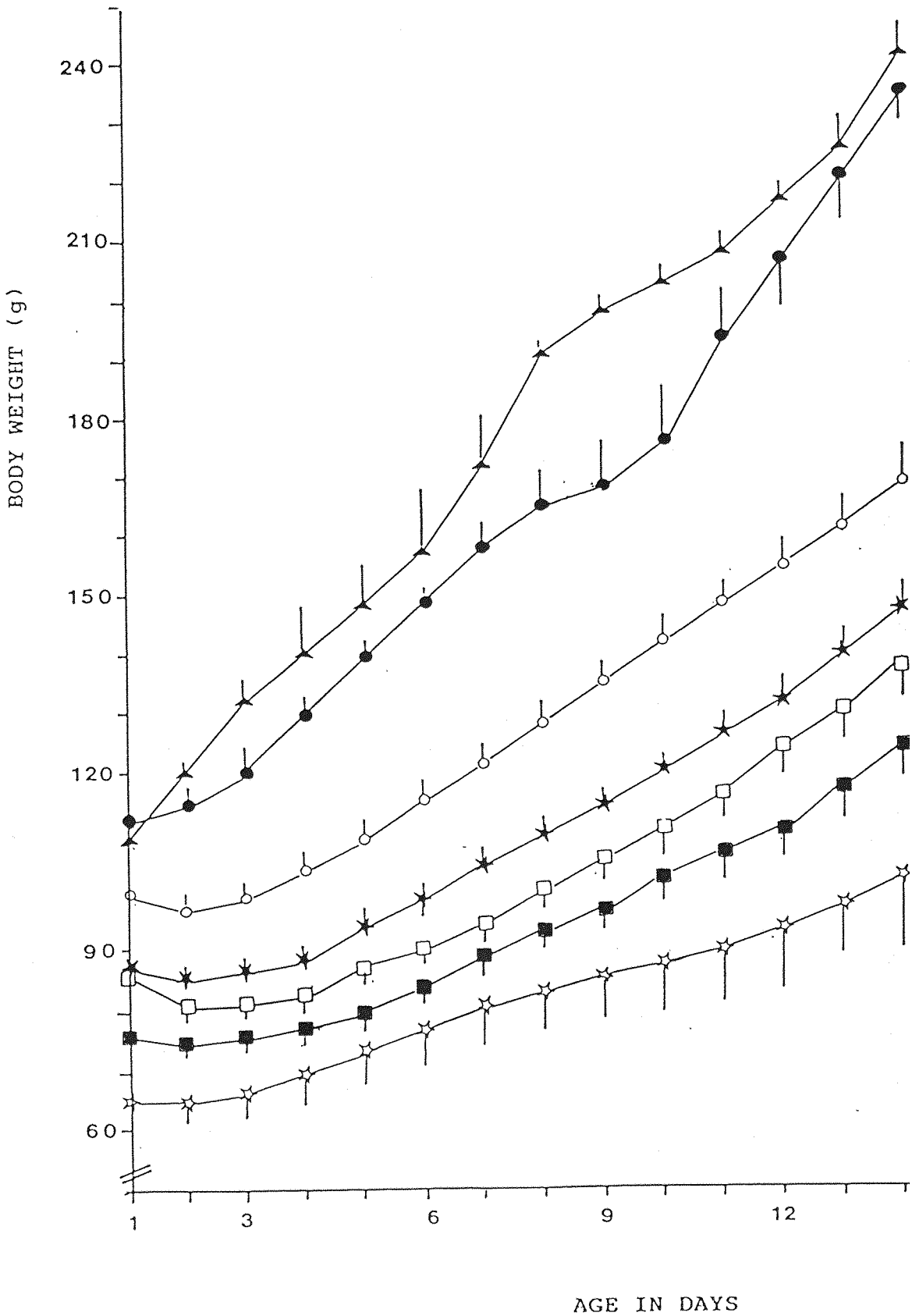


Figure R1-10 The average body weights of neonatal guinea pigs from litters of various sizes. Litters of 1 (▾), 2 (●), 3 (○), 4 (□), 5 (■) and 7 (☆) pups and all groups combined (★).

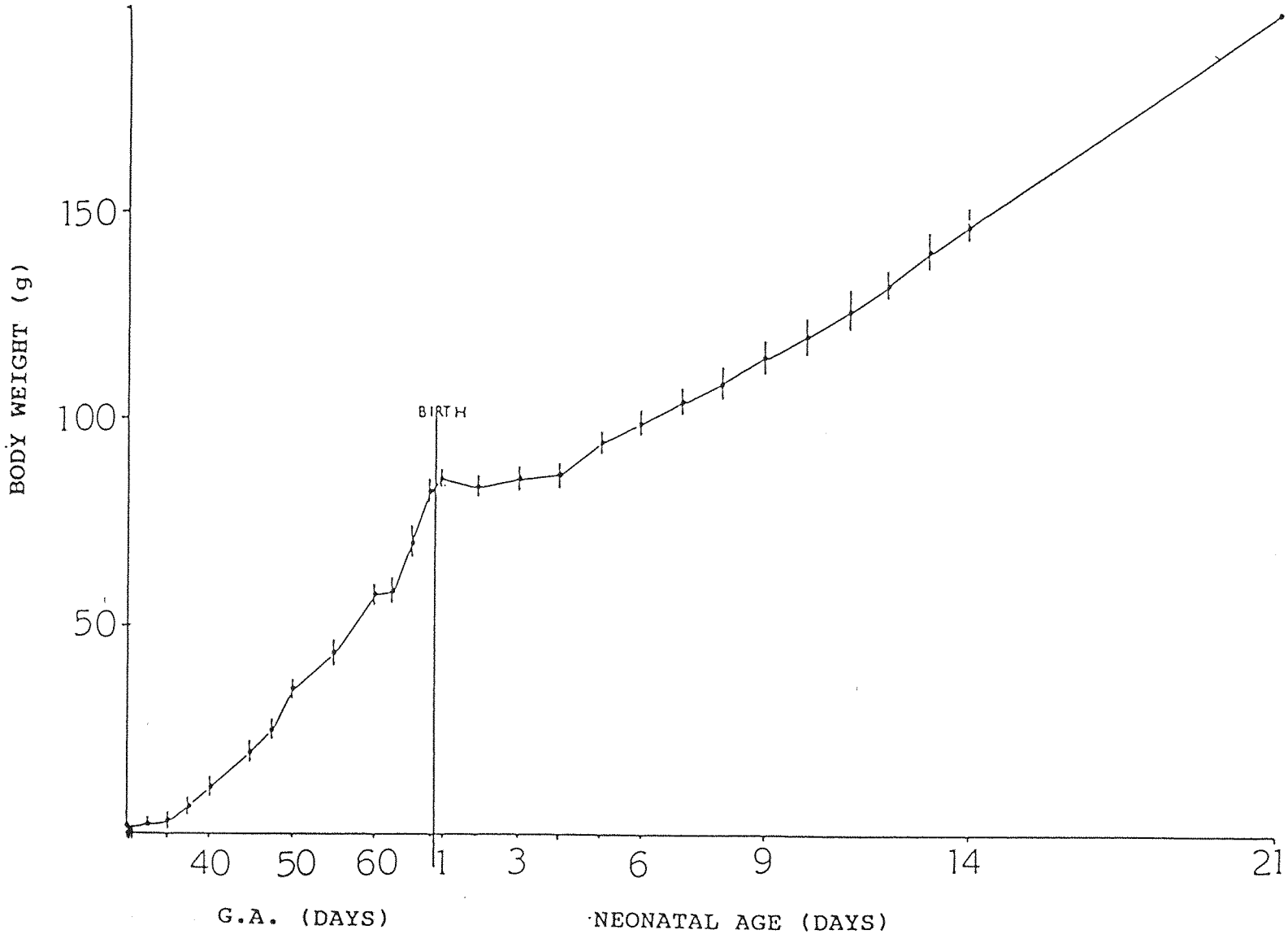


Figure R1-11 The body weight of fetal and neonatal guinea pigs from 30 days gestational age to 21 days post-partum.

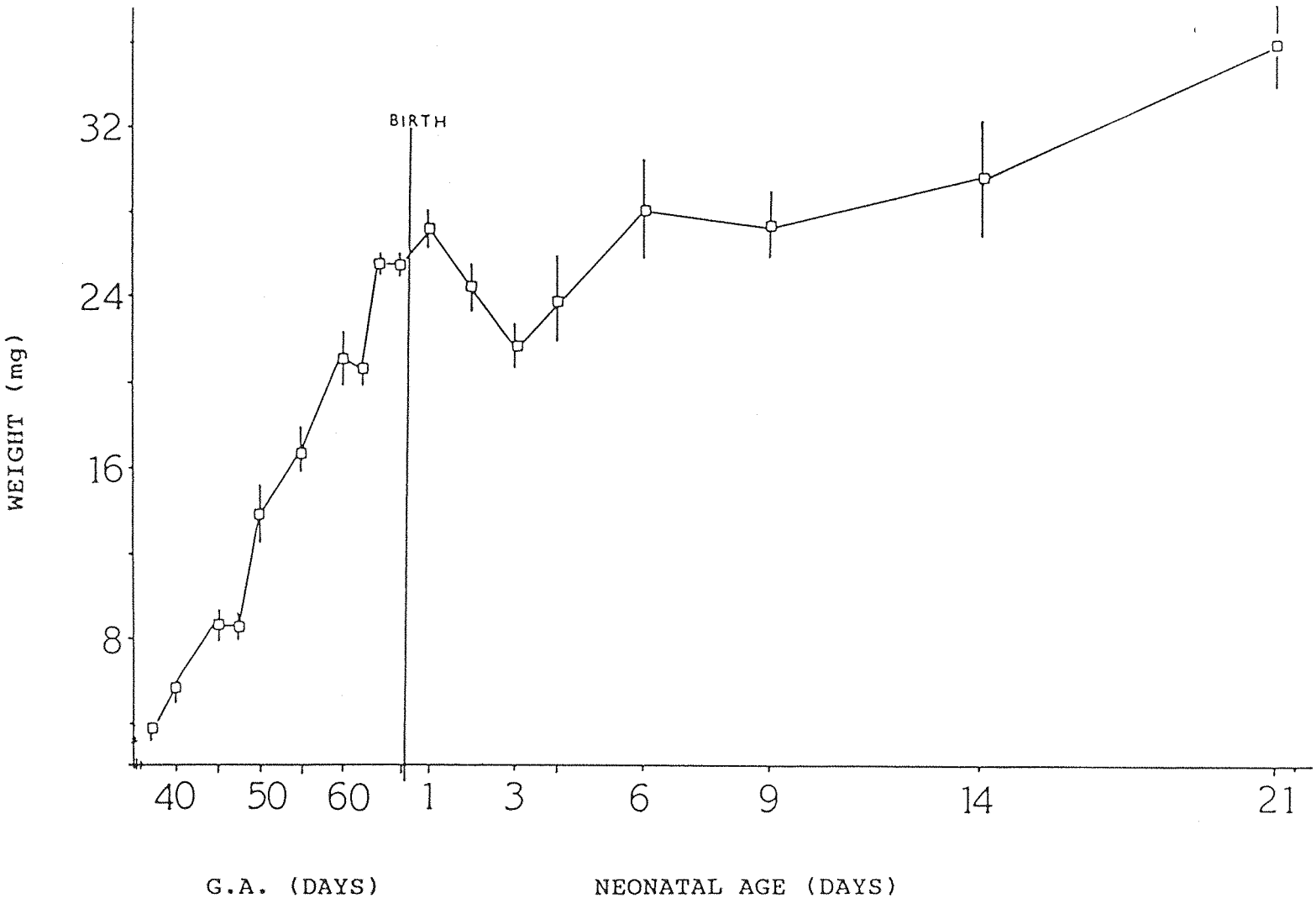


Figure R1-12 The absolute weight of thyroid glands (mg) in fetal and neonatal guinea pigs.

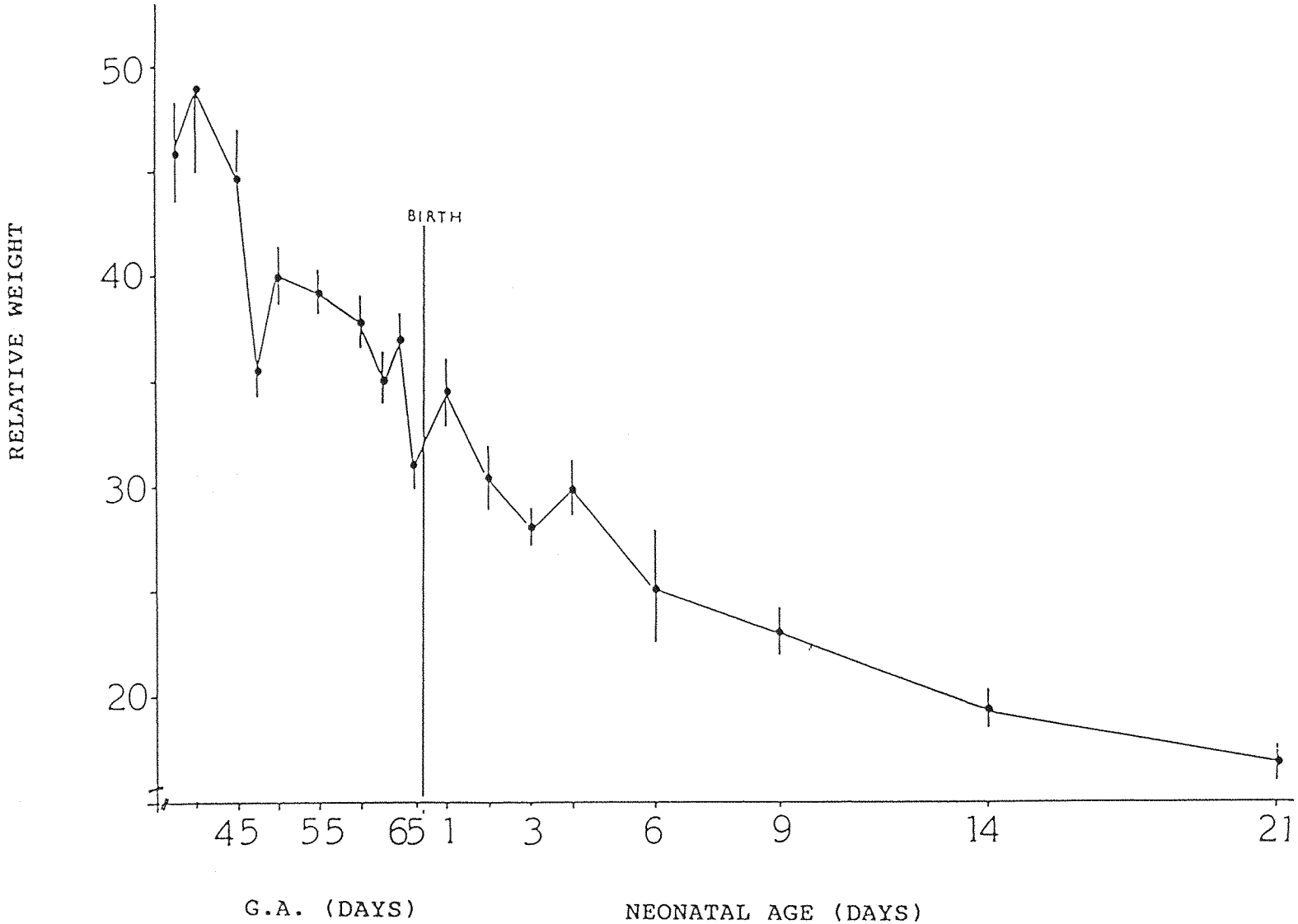


Figure R1-13 The relative weight of the thyroid glands (mg/100 g body weight) in fetal and neonatal guinea pigs.

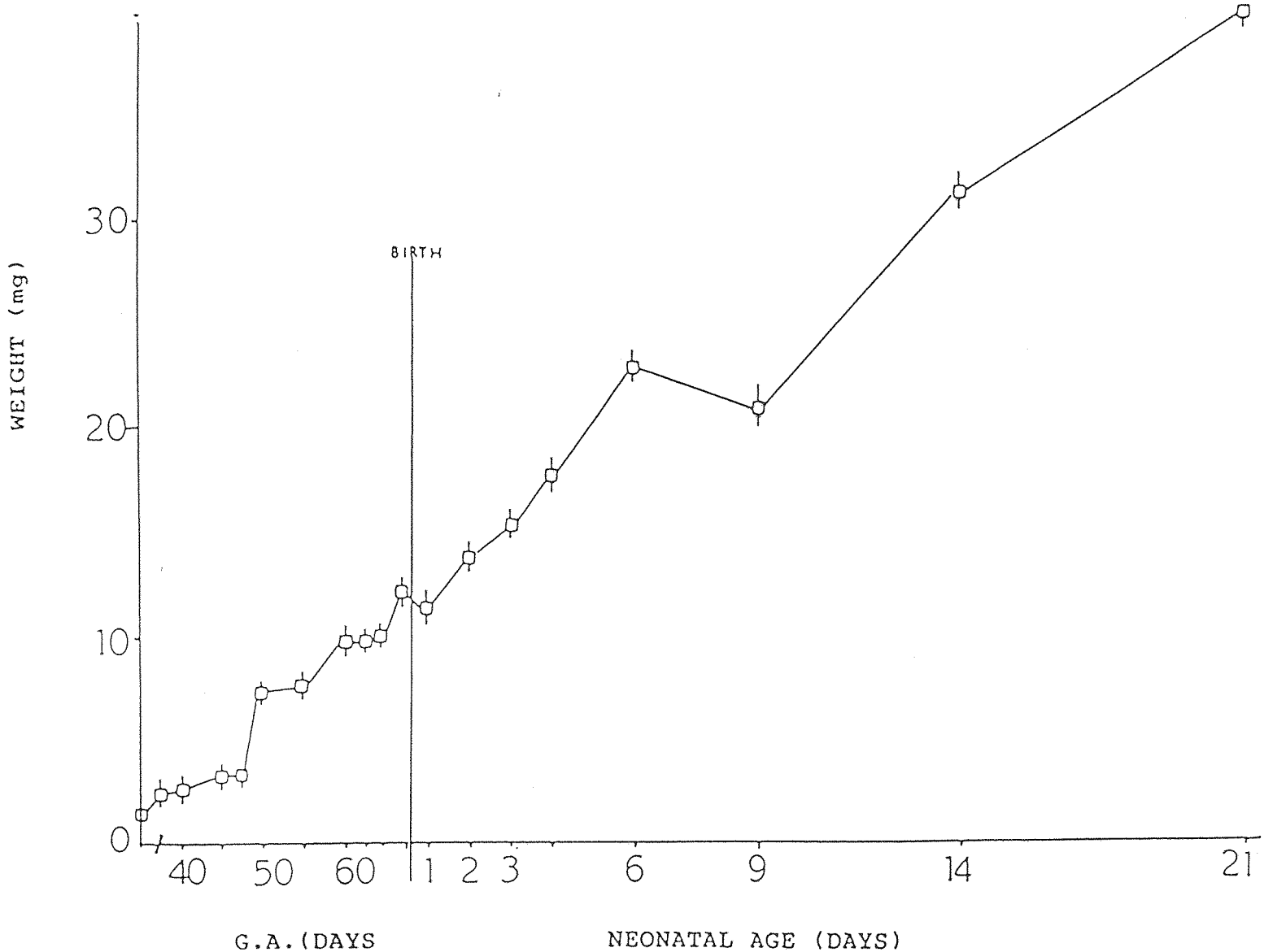


Figure R1-14 The absolute weight of adrenal glands (mg) in fetal and neonatal guinea pigs

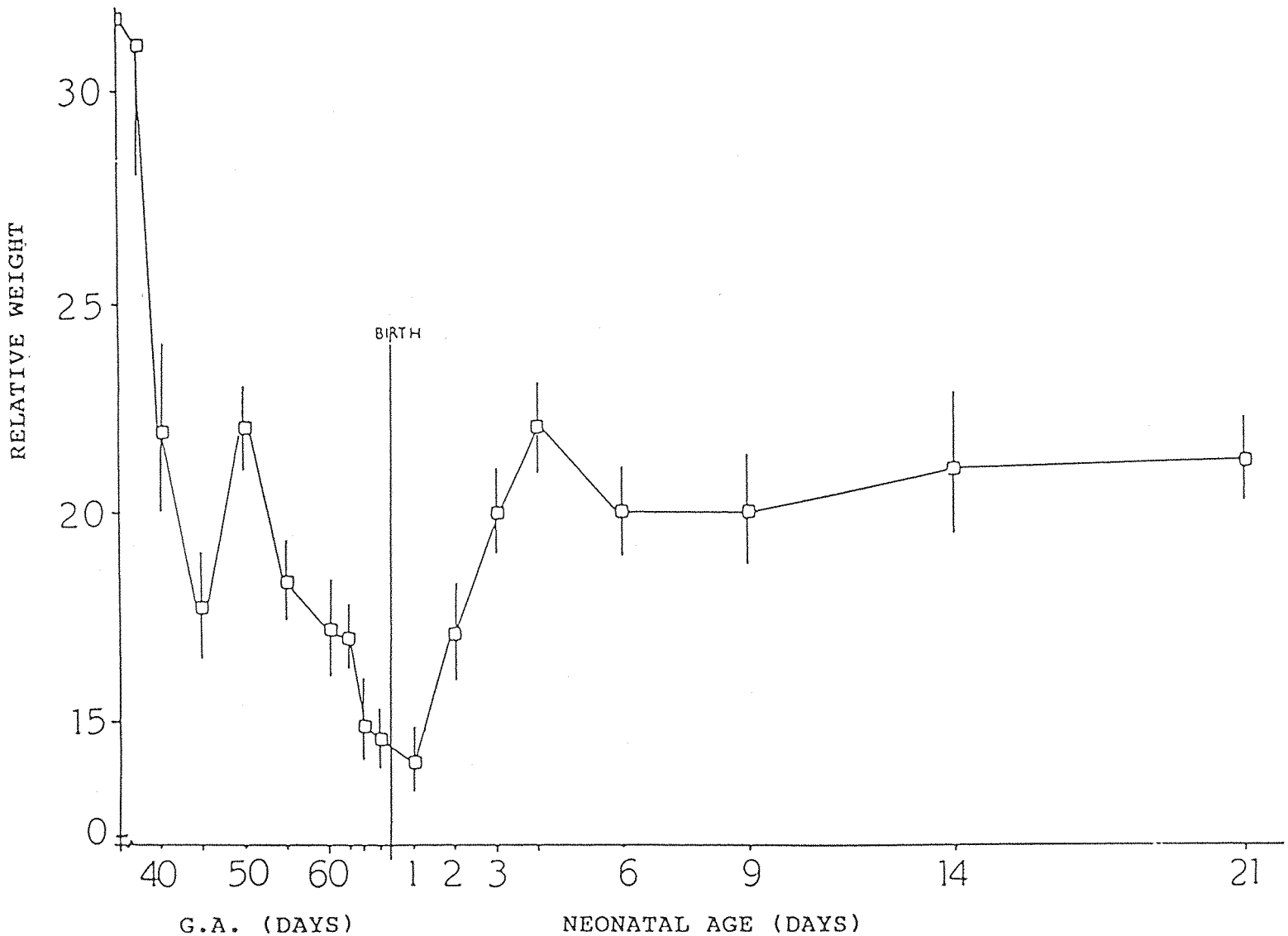


Figure R1-15 The relative weight of the adrenal glands (mg/100g body weight) in fetal and neonatal guinea pigs.

Age in days	Size of Litter 1 (2)	2 (6)	3 (24)	4 (35)	5 (25)	6 (7)	mean (99)
1	109.0	112.0 ± 4.5	99.1 ± 2.5	85.4 ± 1.9	75.8 ± 2.0	64.6 ± 4.1	86.9 ± 1.7
2	120.0	115.0 ± 4.2	96.4 ± 2.7	80.9 ± 1.8	74.2 ± 2.1	64.6 ± 4.2	84.9 ± 1.8
3	132.0	120.0 ± 4.0	98.4 ± 2.7	80.9 ± 1.8	75.4 ± 2.3	65.7 ± 4.4	86.1 ± 1.9
4	140.0	130.0 ± 4.0	103.0 ± 3.0	81.9 ± 2.9	77.0 ± 2.5	69.1 ± 5.4	87.6 ± 2.3
5	148.0	140.0 ± 3.7	108.0 ± 3.0	86.7 ± 2.4	79.6 ± 2.7	72.6 ± 5.8	93.5 ± 2.3
6	157.0	149.0 ± 4.7	115.0 ± 3.3	89.5 ± 2.6	83.2 ± 2.8	76.3 ± 5.9	98.2 ± 2.5
7	172.0	158.0 ± 4.3	121.0 ± 3.4	94.0 ± 2.9	88.3 ± 3.1	79.9 ± 6.4	104.0 ± 2.7
8	191.0	165.0 ± 6.4	128.0 ± 3.7	99.7 ± 3.4	92.8 ± 3.7	82.6 ± 6.9	109.0 ± 3.0
9	198.0	168.0 ± 8.6	135.0 ± 4.0	105.0 ± 3.6	96.5 ± 3.9	85.0 ± 7.5	114.0 ± 3.2
10	203.0	176.0 ± 9.2	142.0 ± 4.5	110.0 ± 3.9	102.0 ± 4.0	87.1 ± 8.3	120.0 ± 3.4
11	208.0	194.0 ± 7.8	148.0 ± 4.9	116.0 ± 4.1	106.0 ± 4.5	89.6 ± 8.9	126.0 ± 3.6
12	217.0	207.0 ± 8.7	154.0 ± 5.3	124.0 ± 4.4	110.0 ± 5.0	93.0 ± 10.3	132.0 ± 3.9
13	226.0	221.0 ± 7.7	161.0 ± 5.6	130.0 ± 4.7	117.0 ± 5.2	97.1 ± 11.3	140.0 ± 4.1
14	242.0	236.0 ± 6.0	169.0 ± 6.0	137.0 ± 4.9	124.0 ± 5.5	102.0 ± 12.6	147.0 ± 4.4

Table R1-4. Growth of male and female guinea pigs from day one to weaning

Age in days	Body wt g	Thyroid wt mg	Thyroid wt mg %	Adrenal wt mg	Adrenal wt mg %
1 (20)	79.1 ± 2.5	27.4 ± 0.9	34.6 ± 1.5	11.3 ± 0.5	14.2 ± 0.8
2 (20)	77.5 ± 1.9	24.3 ±1.3	31.1 ± 2.0	13.5 ± 0.9	17.4 ± 1.0
3 (15)	79.2 ± 2.9	21.8 ± 0.8	27.9 ± 0.8	15.2 ± 2.6	19.4 ± 0.9
4 (16)	79.1 ± 3.6	23.7 ± 1.6	30.0 ± 1.2	17.0 ± 1.0	21.9 ± 1.0
6 (13)	106.6 ± 4.8	28.8 ± 1.5	27.1 ± 2.3	21.0 ± 2.8	20.8 ± 1.0
9 (10)	117.0 ± 6.1	27.3 ± 1.7	23.3 ± 0.9	20.6 ± 1.3	17.6 ± 1.3
14 (10)	153.2 ± 9.8	29.6 ± 2.1	19.5 ± 0.9	31.2 ± 1.5	21.2 ± 1.4
21 (10)	215.1 ± 9.2	36.0 ± 1.9	16.8 ± 0.9	44.8 ± 2.0	21.1 ± 0.8

Table R1-5. Relative (mg/100 gm body weight) and absolute (mg) weight of the thyroid and adrenal glands in normal neonatal guinea pigs.

Results Section 2 - Histology

The Thyroid Gland

Introduction

The study of the fetal thyroid gland was carried out between 30 and 65-66 days of development. The structure of the newborn and adult glands was also investigated.

Results

The thyroid gland is composed of two lobes, both parts lie alongside the trachea and are connected by a narrow isthmus. The gland in the fetal guinea pig has a compact structure. The gland is covered by an outer connective tissue capsule. Under this there is an inner capsule which is thinner and adheres closely to the gland. Continuations of the inner capsule extend as septa into the gland dividing it into indefinite lobes and lobules. The gland is infiltrated by a rich network of capillaries filled with erythrocytes which are often clumped together in large masses.

The follicle, the structural unit of the gland, is defined as a completely closed sac whose wall is usually made up of only a single layer of epithelial cells and which contains a colloidal mass of viscous protein solution. The size and shape of follicles may vary and differences are found in these respects in follicles of the same gland as well as in follicles of fetuses at different stages of development.

Observations

The guinea pig thyroid does not develop synchronously throughout gestation. Generally the centre of the gland shows a more advanced stage of maturation. The average diameter of the follicles is shown in Figure R2:1 which also serves to show the change in follicular size as development proceeds.

It is helpful to divide the maturation of the thyroid gland into three phases :-

a. The precolloid stage which, in this study was found to be up to 30 days.
b. The period during which active colloid formation commenced.
c. The period of follicular growth.

a) The precolloid stage The thyroid gland in 30 day fetuses was found to be made up of a solid mass of epithelial cells. It was not possible to dissect out the gland for histological study at this age, but section of the entire neck region showed the thyroid gland to consist of a network of anastamosing dense cords of cells. At this stage the fetal thyroid does not accumulate iodide .

b) The initial colloid formation The most characteristic part of this stage of development was the initial appearance of colloid amongst the cells. The normal structure of the thyroid consisting of colloid filled follicles is established by days 34 to 35 of gestation (Figure R2-2 and R2-3). The first follicles make their appearance in the centre of the gland where a few follicles are filled with a weakly staining colloid. The follicles are surrounded by cuboidal epithelial cells. The cytoplasm of the follicular epithelial cells is faintly orange and the nuclei are stained intensely. Connective tissue and groups of erythrocytes appear between the follicles. The size of the follicles varied between 8-25 μ in diameter (average diameter 10.3 ± 1.5μ). At this stage the first evidence of iodide trapping by the gland can be detected .

c) Follicular Growth

38 Days

There is increasing storage of colloid within the follicles as well as an increase in follicular number and diameter, the diameter of the follicles reaches 20.8 ± 1.22 μ (range 10-30μ). They are placed

irregularly with respect to one another. The cells of the follicular epithelium are cuboidal (Fig. R2-4).

40 Days

The increase in the volume of the thyroid gland is accompanied by an increase in both the number and size of the follicles. The follicular size is variable (average 26.4 ± 1.1μ ; range 20-35μ) and they are generally round or oval in form.

45 Days

By 45 days the number of follicles has increased considerably and they are also showing some changes in both size and form. At this stage the follicular diameter ranges from 20 to 45 microns (mean 31.1 ± 1.3μ) and the follicular shape is more uniformly circular. The connective tissue septa are still apparent and and the follicles in the centre of the gland are larger than those in the periphery.

50 Days

The number of circular follicles continues to increase a few follicles are now lined by low, columnar epithelial cells. The mean follicular diameter was 34.2 ± 1.7μ (range 20-50μ)

55 Days

A study of sections at this stage shows numerous follicles surrounded by columnar epithelial cells whose cytoplasm is more orange and whose nuclei are more lightly stained. The number of irregular shaped follicles is now much less. There are however a few solid inter follicular epithelial cell masses still present at this stage. The diameter of the follicles ranged from 10 to 50 μ (mean 34 ± 1.7μ) and the colloid which they contain is more homogeneous(Fig. R2-5).

60 days

Circular follicles make up almost the whole of the tissue by this

age. The follicles are closely packed and separated by delicate connective tissue. There is more columnar epithelium present and the nuclei are stained lightly whilst the cytoplasm is stained an intense orange(Fig. R2-6). The average follicular diameter is 52 ± 4.28 µ (range 30-100 µ).

62 Days

There is great variability in the size of the follicles by this age. The largest follicles now being distributed throughout the gland. The diameter of the large follicles is about 130µ whilst that of the small follicles is about 20 µ (mean 66.2 ± 3.3µ). Most are circular in form but a few are irregular (oval or spindle shaped). The follicles are more closely packed and lined by columnar epithelium. In the centre of the gland there is an area of papillary infolding of columnar epithelium.

64-66 Days

The structure at this stage is generally the same as that at 62 days but the size of the papillary infolding of columnar epithelium at the centre of the gland is increased and smaller patches appear in other areas. Numerous colourless spherical follicles are clearly seen with excessive accumulation of colloid. Many follicles are over distended with colloid. The diameter of the larger follicles is about 190 µ whilst the smallest follicles are only about 10 µ (mean 95 ± 4.7 µ).

One Days old

The whole picture is one of an active gland. The areas of papillary infolding columnar epithelium form about one third of the whole gland whilst the size of the large follicles is about 135 µ (mean 82.66). The follicles have variable shape and a number contain no colloid, others are filled with homogeneous colloid and many others contain colloid which is interspersed with a number of vesicles (Fig. R2-7).

Normal Adult Thyroid

The follicles are closely packed and surrounded by flatter epithelial cells with slightly flattened nuclei which stain faintly and the cytoplasm stains orange. The follicles contain homogeneous purple coloured colloid.

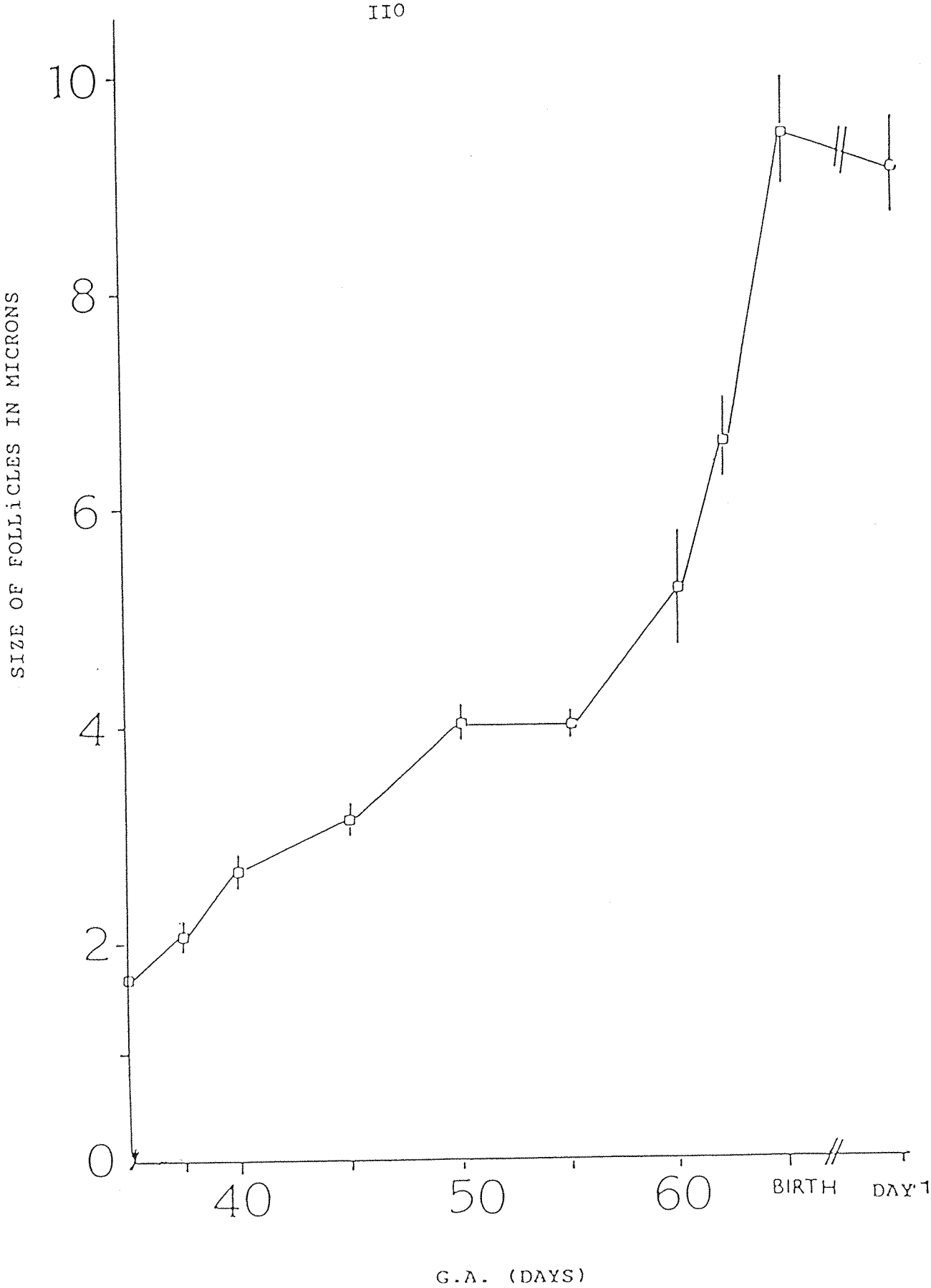


Figure R2-1 The change in the average diameter of thyroid follicles as fetal development proceeds.

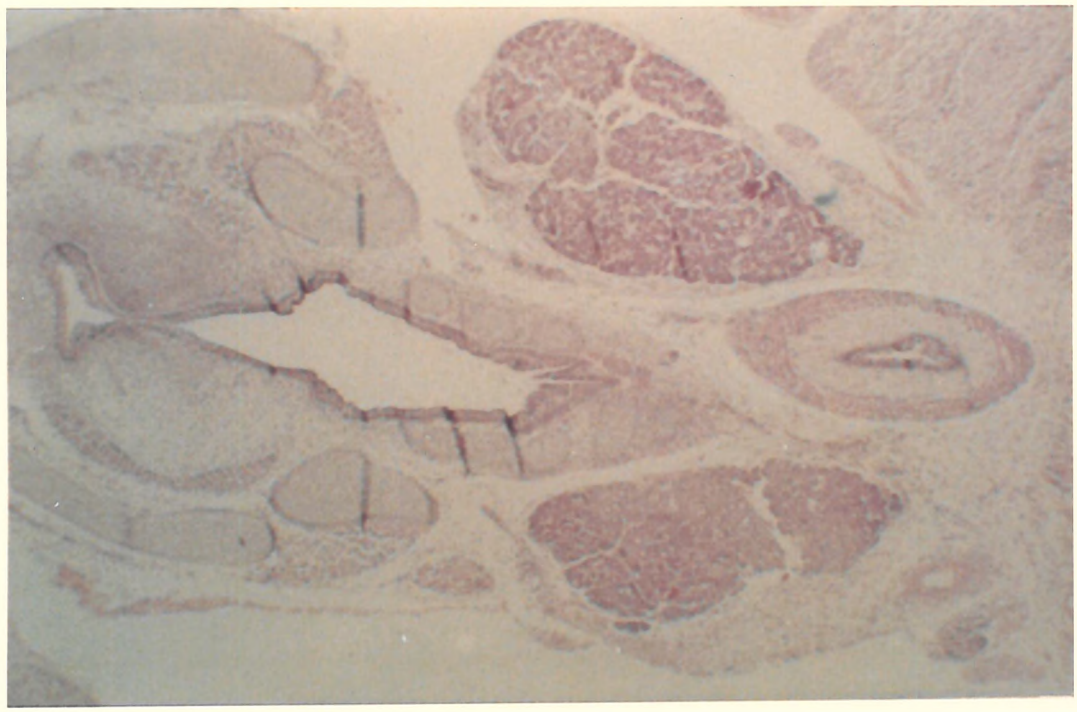


Figure R2-2 Transverse section through part of the neck region to show the thyroid gland in a 35 day guinea pig fetus (H&E. X 25).

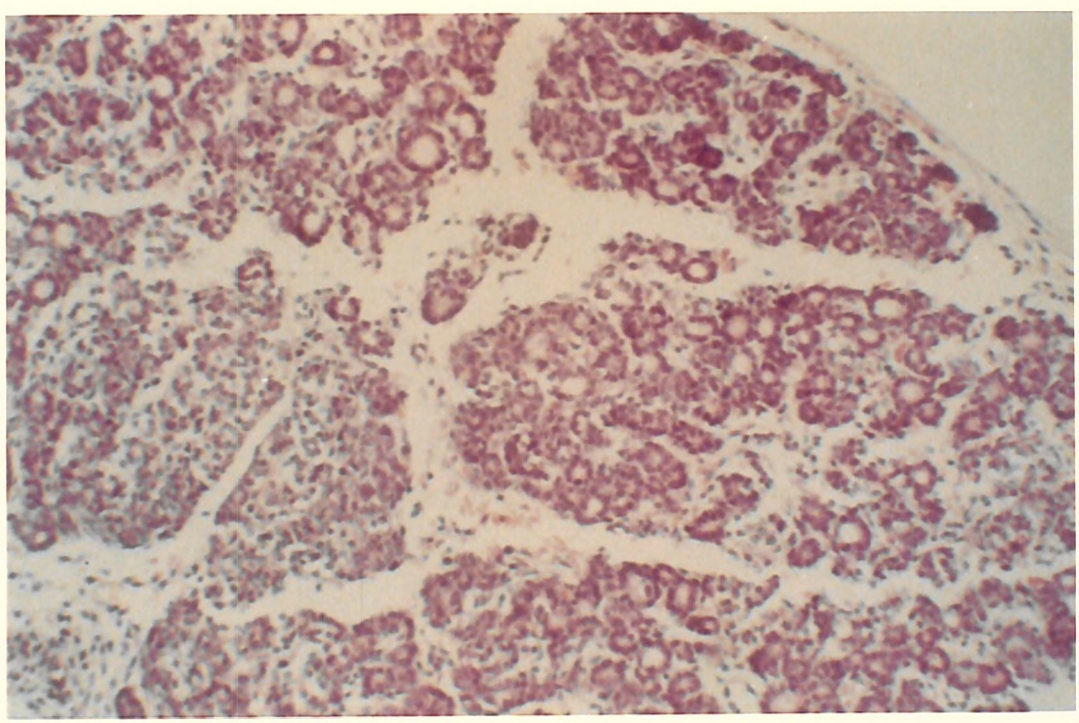


Figure R2-3 Thyroid gland of a guinea pig fetus at 35 days gestational age (H&E. X 96).

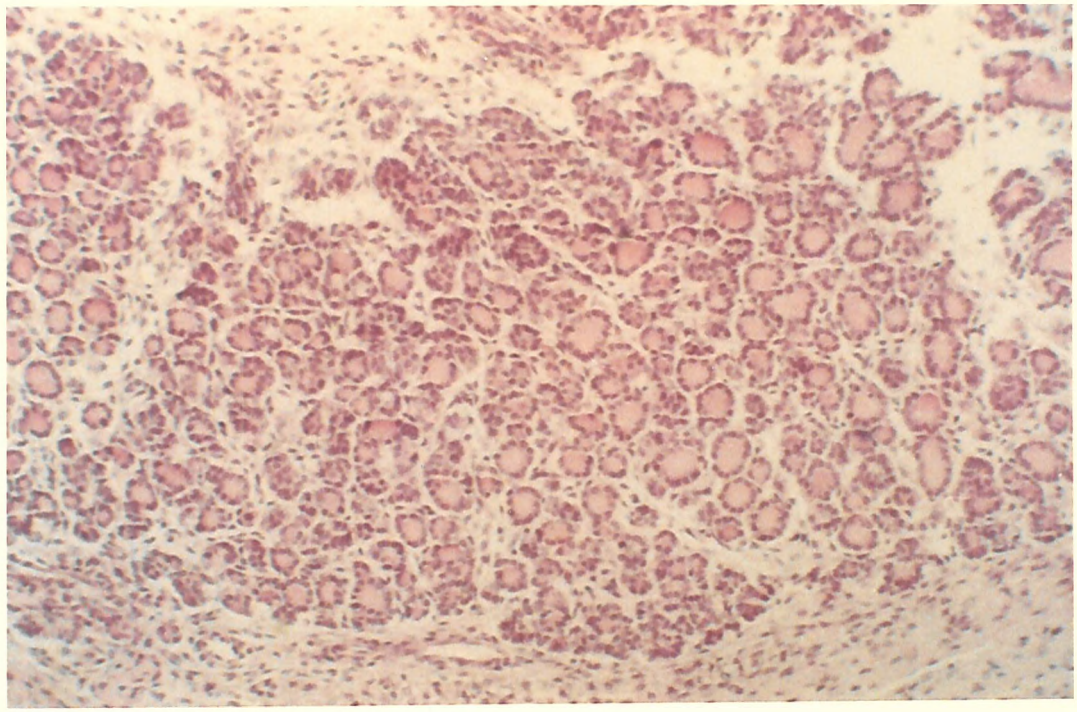


Figure R2-4 Thyroid gland of a guinea pig fetus at 38 days gestational age (H&E. X 96).

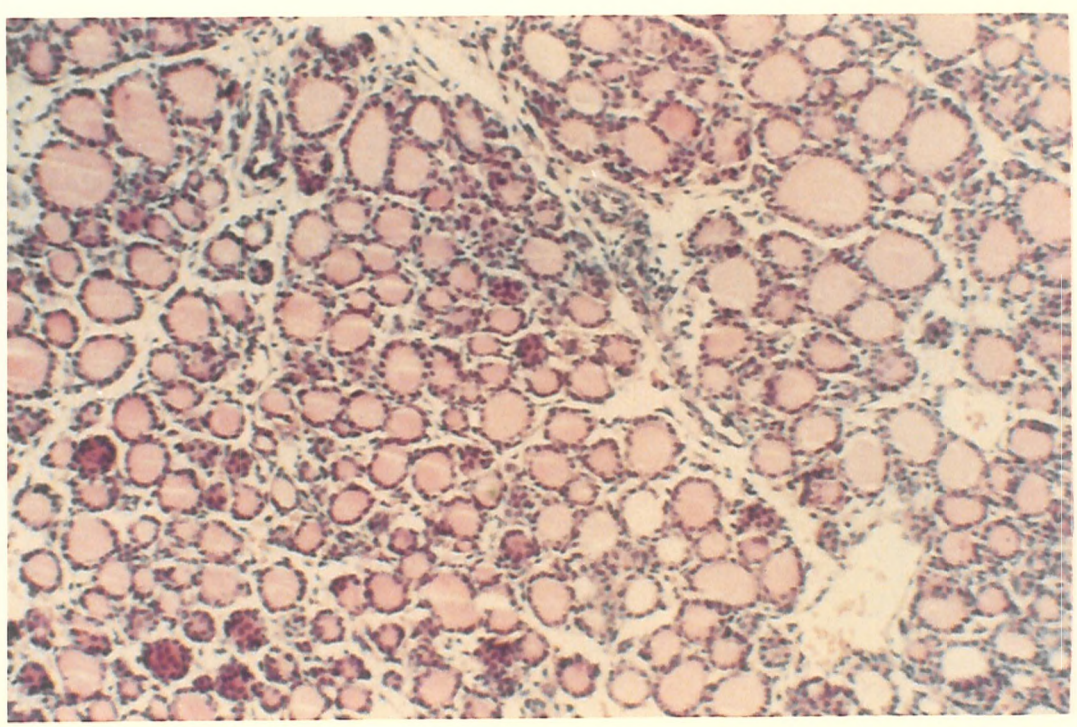


Figure R2-5 Thyroid gland of a guinea pig fetus at 55 days gestational age (H&E. X 96).

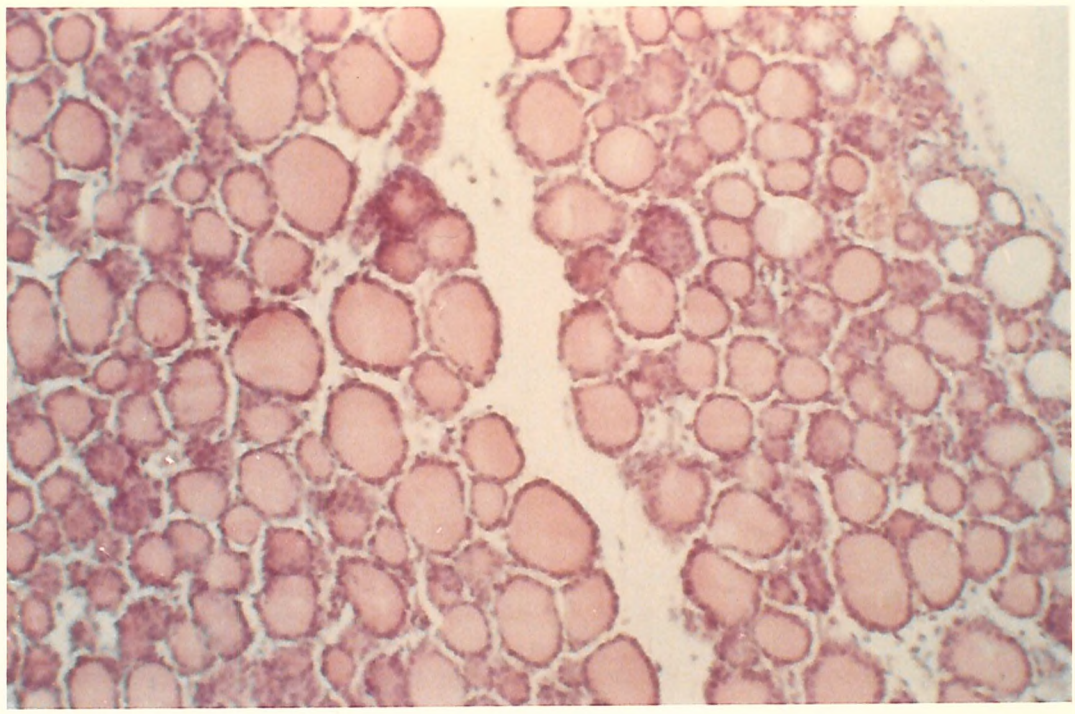


Figure R2-6 Thyroid gland of a guinea pig fetus at 60 days gestational age (H&E. X 98).

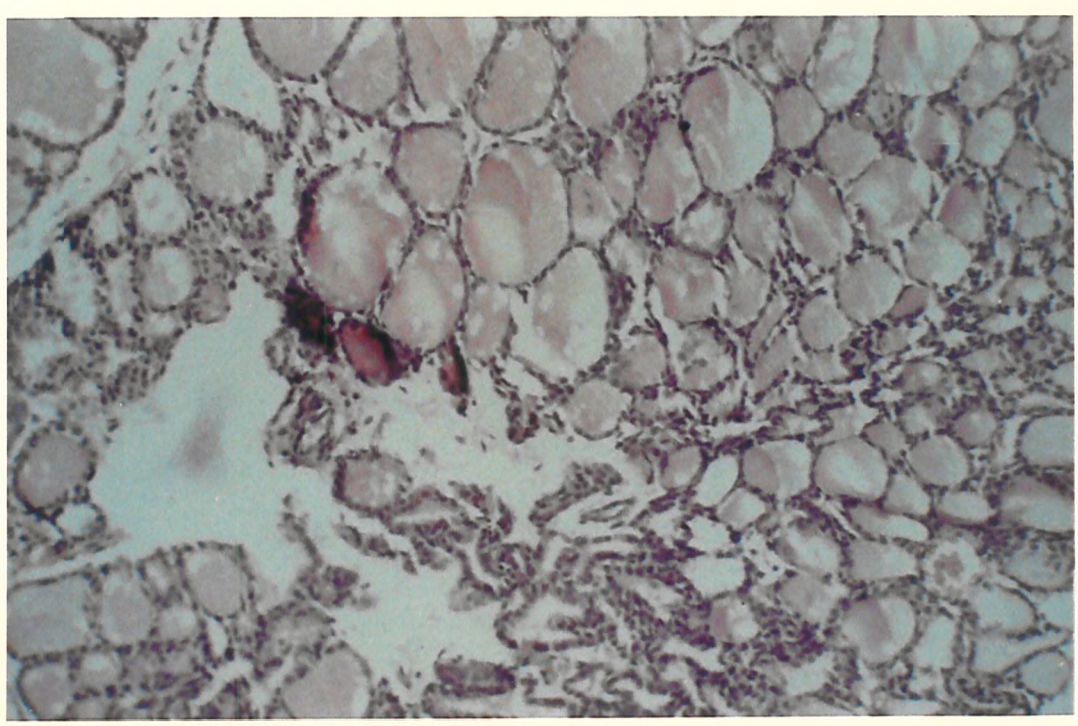


Figure R2-7 Thyroid gland of a 24 hour-old neonatal guinea pig. (H&E. X 96).

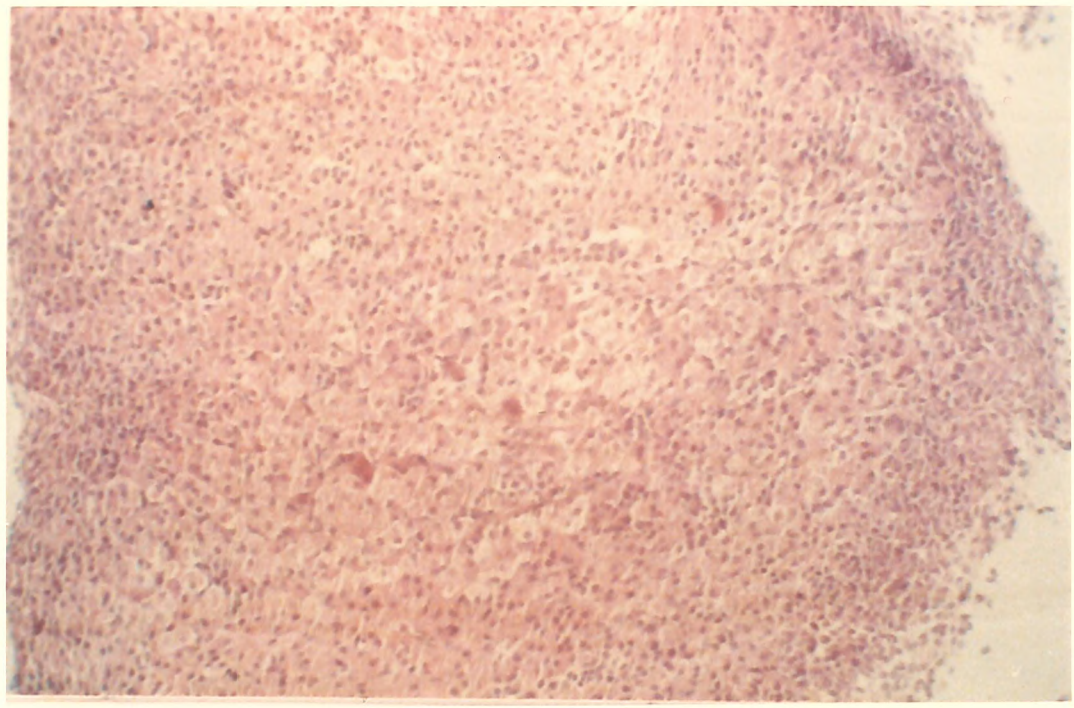


Figure R2-8 Adrenal gland of a guinea pig fetus at 35 days gestational age (M.H. X 80).

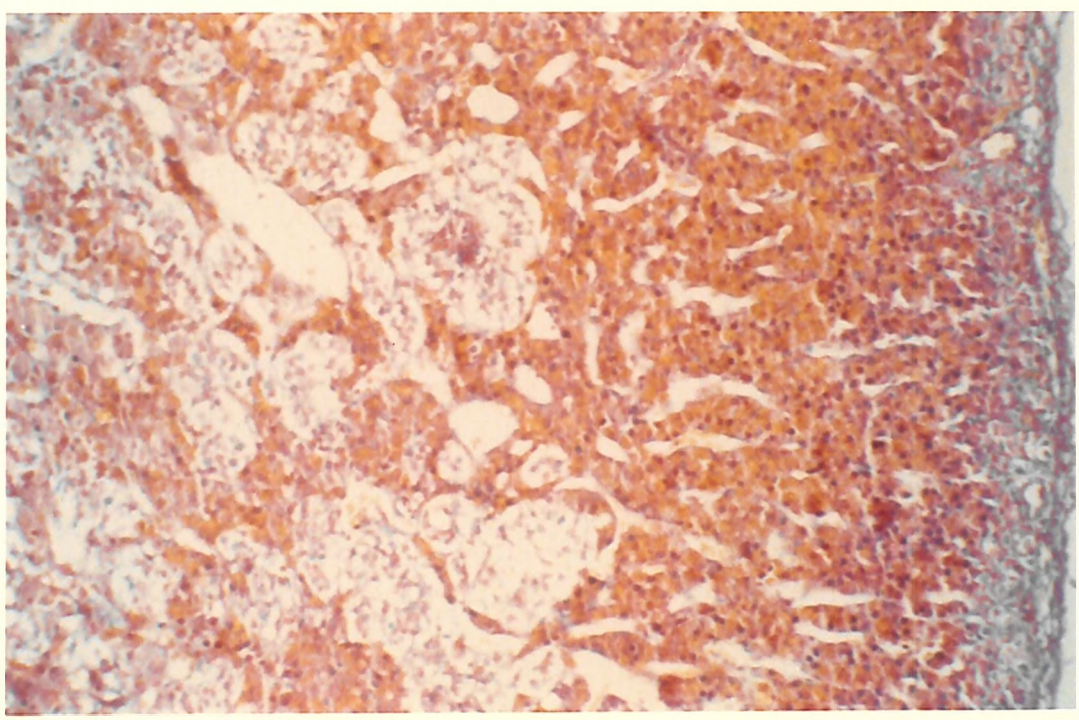


Figure R2-9 Adrenal gland of a guinea pig fetus at 50 days gestational age (M.H. X 84.8).

Adrenals

Introduction

The adrenal glands are flattened organs one lying at the pole of each kidney. A thick connective tissue capsule surrounds the gland and contains small blood vessels. A sectioned gland shows two regions an outer cortex and inner part, the medulla, which produces adrenaline and noradrenaline. The cortex, the major part of the gland, is divided into three layers. The outer zone, the zona glomerulosa, consists of small columnar epithelial cells with deeply staining nuclei and secretes mineralocorticoids. The thicker innner zone, the zona fasciculata, is the widest portion of cortex and consists of large irregularly cuboidal cells. The cells, which appear vacuolated, produce and secrete cortisol and these cells are normally regulated by ACTH. The inner zone, the zona reticularis, is similar to the zona fasciculata. These cells lie adjacent to the medulla and secrete mainly sex hormones.

It has been reported that cells isolated from the zona reticularis have low basal production of progesterone and cortisol and that there is no steroid response to ACTH (Nishikawat & Strott, 1984; Obara, Mikami, and Strott,1984).

Observations

Normal Adult

In the adult the above morphological zonation can be seen macroscopically if the gland is sliced in the midline along the axis. The gland is surrounded by a thin reddish brown coat (capsule and glomerulosa) and a thick yellow zone, the zona fasciculata, which contains lipid rich cells and occupies about 40% of the cortex. A dark red/brown gelatinous zone, the zona reticularis, extends to the medulla in the centre of the gland.

Fetal

The histogenesis of the adrenal cortex in the fetal guinea pig ranging from 35 days to term was examined by light microscopy. The adrenal glands from neonates were also examined.

35 days

At 35 days of intrauterine life the differentiation of cortical cells gives rise to two histologically distinct regions. The outer zone, the glomerulosa cells, are arranged in ball like clusters (5-7 cells per cluster). The cells within the inner zone are composed of large rounded or oval cells with dark stained nuclei (Fig. R2-8).

40 days

The zona glomerulosa is composed of clusters of cells (about 7-10 cells per cluster) inside the connective tissue capsule. These cells characteristically have a high nucleus/cytoplasma ratio. The cells of the inner zone are also increased in number with dark stained nuclei and pale cytoplasm.

45 days

Blood vessels are clearly seen inside the capsule among the zona glomerulosa cells. The cells of the inner zone are arranged in cords about 20-25 cells long.

Mallory-Heidenhaim (M & H) Stain

The above observations were made using a haemotoxylin and eosin stain. Using an M & H stain at 50 days the fetal adrenal cortex could be divided into three regions. The outer zone and the middle zone were similar to the zona fasciculata of the adult. The cells were vacuolated with large dark nuclei and the layer is about 25 to 30 cells thick while the inner zone, the zona reticularis, has cells which are larger in size with dark brown/red cytoplasma and is about 10 to 20 cells thick (Fig. R2-9).

Between 50 and 62 days of gestation the adrenal gland grows rapidly

and slicing the tissue using the microtome is not easy due to the increasing number of lipid rich cells in the zona fasciculata. At 55 days the inner zone (fasciculata and reticularis) was about 40 cells thick and more tightly packed with the zona glomerulosa. The cells of the inner zone appear vacuolated (clear cells) due to the loss of lipid which occurs during the histological processing.

64 days to term (H and E stain)

The three zones can clearly be seen at day 64. The middle zone cells are arranged in columns extending from the zona glomerulosa to the reticularis, about 30-40 cells long while the cells of the inner zona reticularis have relatively lipid sparse cytoplasm with dark nuclei and are arranged in cords about 10-20 cells long.

One day old

The three zones are clearly seen. The zona glomerulosa cell clusters now have 10-15 cells/cluster. The cells of the zona fasciculata are usually vacuolated and form a layer about 40 cells thick while the inner, zona reticularis, is about 20 cells thick.

Anterior Pituitary

Introduction

Investigations were conducted into the development of the anterior lobe of the pituitary in the guinea pig fetus starting at 40 days of intrauterine life and ending with newborn animals.

Observations

On histological examination the anterior lobe is seen to take the form of anastomosing cords and masses of cells supported by a network of connective tissue and blood vesels. The anterior lobe of the guinea pig pituitary is composed of two basic cell types, chromophobes and chromophils.

i. Chromophobes The most numerous anterior pituitary cells (the chief cells) are scattered throughout the anterior lobe occupying predominantly the central part of the parenchyma. They are small, rounded cells. The cell boundaries are not easily visible. Chromophobe cells are divided into two types those precursor cells which give rise to any cell type in the anterior pituitary and those which secrete ACTH.

ii. Chromophils Chromophil cells may be subdivided into acidophils and basophils. The most numerous cells are the acidophils which are concentrated mainly in the lateral part of the anterior lobe. They are of various forms but are mainly oval or rounded and their cell boundaries are distinct. The cytoplasm is rich in granules which take up Orange G stain. These cells secrete growth hormone. The basophil cells are larger than the acidophils. They stain poorly with H & E but are stained deeply by the M & H stain and secrete TSH. The cytoplasmic granules are less numerous and are smaller. The cell boundaries are clear and distinct. The anterior lobe of the pituitary is separated from the intermediate lobe by the hypophysial cleft. The cells of the intermediate lobe on the side

bordering on the posterior lobe frequently have basophil granules in the cytoplasm.

The anterior lobe of the pituitary of the fetal guinea pig at 40 days is in the form of irregular cords and masses of cells. These cells are packed close to each other. Chief cells are the most numerous cells at this stage few eosinophils or acidophils are present. In 45 day old fetuses the anterior lobe cells absorb the stain more intensely. It is possible to distinguish acidophil cells which are concentrated mainly in the lateral part of the anterior lobe. They are small in size. Basophils are also small cells which stain deeply. They are concentrated in the anterior region and extend laterally into the anterior lobe. By 50 days the number and size of the cells has increased considerably. Acidophils are more numerous than basophils. Acidophils, basophils and chief cells are arranged in anastomosing cords in the anterior lobe parenchyma. At 55 days the increase in the volume of the pituitary gland is accompanied by an increase in both the number and size of basophils and acidophils. Acidophils and basophils are scattered throughout the anterior lobe. More basophil cells appear in the inner intermediate lobe. From 60 days to term the number and size of these basophil cells increase as the fetal period comes to an end. Acidophils appear mainly in the lateral border of the anterior lobe and they are also found in the parenchyma.

Results Section 3 - Cortisol

Introduction

Considerable species differences have been observed with regard to the type of hormone secreted by the adrenal cortex. Among the common small laboratory animals the guinea pig has an adrenal hormone pattern most closely approximating that in man. The development of adrenocortical secretion in the fetus, neonate and in the pregnant female guinea pig has been studied.

Cortisol response to anaesthesia

Table R3-1 shows the differences between maternal and fetal plasma cortisol levels obtained from two groups of pregnant guinea pigs (i) sampled after anaesthesia and (ii) killed by a blow to the neck. Generally, after anaesthesia, the plasma cortisol value measured in the mothers was higher than that of the corresponding cortisol value obtained from non-anaesthetised pregnant guinea pigs, throughout gestation. In the fetuses this difference only appeared 3 to 4 days before term.

No significant difference was observed in the cortisol concentration from 50 to 60 days of gestation in the fetal plasma obtained from the two groups. By 64 days the fetal plasma cortisol concentration measured in fetuses from anaesthetised mothers had increased significantly ($P < 0.0001$) to a value of about twice that of corresponding cortisol levels in the fetal plasma obtained from non-anaesthetised mothers. At term the cortisol concentration was 1030 ± 90 ng/ml in the fetuses obtained from anaesthetised mothers significantly higher ($P < 0.0001$) than that of corresponding value measured in the fetal plasma obtained from non-anaesthetised mothers (356 ± 29 ng/ml). Only a small, non-significant, difference was found between the term fetal plasma cortisol concentration in

	Mother	Fetus	Mother	Fetus
	35 days		40 days	
1	840± 60 (3)		1400 (2)	
			*	
2			2580±199 (3)	
3		1820±352(3)		1040±196(8)
	45 days		50 days	
1	1480 (2)		1630±146 (3)	90±22 (6)
			**	
2	3000±80 (3)		3380±277 (3)	150 (1)
3		932±123(6)		612±72(11)
	55 days		60 days	
1	2300±560 (3)	160±13 (7)	3110±153 (4)	245±18(12)
2	3900±265 (3)	200±18 (4)	3970±233 (3)	278±40 (5)
3		806±63 (12)		1470±119 (11)
	62 days		64 days	
1	3550 (2)	307±38 (8)	3750 (2)	339±15 (8)
	*	*	*	****
2	4500 (2)	339±24 (4)	5290 (2)	740±20 (3)
3		863±95 (7)		605±117(3)
	65-67 days			
1	4410 (2)	356±29 (9)		
		****		
2	5900±326 (3)	1030±90 (5)		
3		348±15 (6)		

Table R3:1. Plasma cortisol level (ng/ml) in pregnant guinea pigs and their fetuses from day 35 to term and fetal adrenal gland cortisol concentrations (ng/100 mg gland).

(1) Mothers killed by below to the neck.

(2) Mothers killed after anaesthesia.

(3) Fetal adrenal gland cortisol concentrations.

anaesthetised animals and the concentration of plasma cortisol of the newborn immediately after birth. While a significant ($P < 0.0001$) difference was found between the value observed immediately after birth and value obtained just before term from fetuses obtained fron non-anaesthetised mothers.

The mean values of maternal plasma cortisol in the anaesthetised groups were higher at 40 and 45 days than those of corresponding non-anaesthetised mothers. At 50 days the difference in plasma cortisol levels between the two groups was significant ($P < 0.01$). No significant differences were found between the two groups from 55 to 60 days of gestation. But after combining the plasma cortisol measurements made at 55 and 60 days in anaesthetised mothers (3933 $\pm$ 159 ng/ml; n=6) and comparing them with the corresponding values measured in non-anaesthetised mothers (2792 $\pm$ 206 ng/ml; n=5) it was found that the anaesthetised values were significantly greater ($P < 0.01$). At 62 days the plasma cortisol concentration in the mothers killed after anaesthesia was greater than that of corresponding cortisol values obtained from mothers killed by a blow to the neck. As at 55 and 60 days if the data obtained at 64 days and term are combined the plasma cortisol concentration in non-anaesthetised mothers is found to be 4043 $\pm$ 240 ng/ml (n=4) and in anaesthetised mothers it is 5486 $\pm$ 250 ng/ml (n=5). The difference is significant ($P < 0.01$).

Changes in maternal plasma cortisol concentration

a. After anaesthesia.

Maternal plasma cortisol concentration increased significantly ($P < 0.05$), from 2580 $\pm$ 199 ng/ml at 40 days to 3900 $\pm$ 265 ng/ml at 55 days of gestation. No changes were found in the mean value from 55 to 60 days. Between day 60 and 64 the plasma cortisol concentration increased significantly ($P < 0.05$) from 3970 $\pm$ 233 ng/ml to 5290 $\pm$ 90 ng/ml. After that the plasma cortisol concentrations remained very high until the last day of pregnancy when

the mean value was significantly higher ($P < 0.001$) than that of day 60 (Fig. R3-1).

b. Without anaesthesia.

Three phases can be seen in the changes in plasma cortisol concentration from 35 days towards term. (i) From 35 days the mean value (840 ± 56 ng/ml) increased to 1400 ± 183 ng/ml on day 40. (ii) Between day 40 and 50 the values were stable. (iii) There was a sharp rise from day 50 to term. During this phase the plasma cortisol concentration increased about three fold from 1630 ± 146 ng/ml on day 50 to 4410 ± 281 ng/ml at term. The increase between day 50 and day 60 was significant at the $P < 0.01$ level whilst that between days 60 and 66 was significant only at the $P < 0.05$ level (Fig. R3-1).

Changes in fetal plasma cortisol concentration

a. After anaesthesia

A rise in fetal plasma cortisol concentrations occurred between days 50 and 55. There was a significant ($P < 0.01$) increase between day 55 and 62 days. The cortisol concentrations then increased progressively to reach a value of 1030 ± 90 ng/ml at term which was significantly greater ($P < 0.05$) than the value of 740 ± 20 ng/ml at day 64 (Table R3-1 and Fig. R3-2).

b. Without anaesthesia

A significant ($P < 0.05$) change occurred in fetal plasma cortisol concentration between days 50 and 55 of gestation and ($P < 0.01$) between days 55 and 60. The difference between days 60 and 62 was not significant but between day 60 and 64 the increase was significant ($P < 0.01$). The highest levels of fetal cortisol were measured at term (356 ± 29 ng/ml; Fig. R3-2 and Table R3-1).

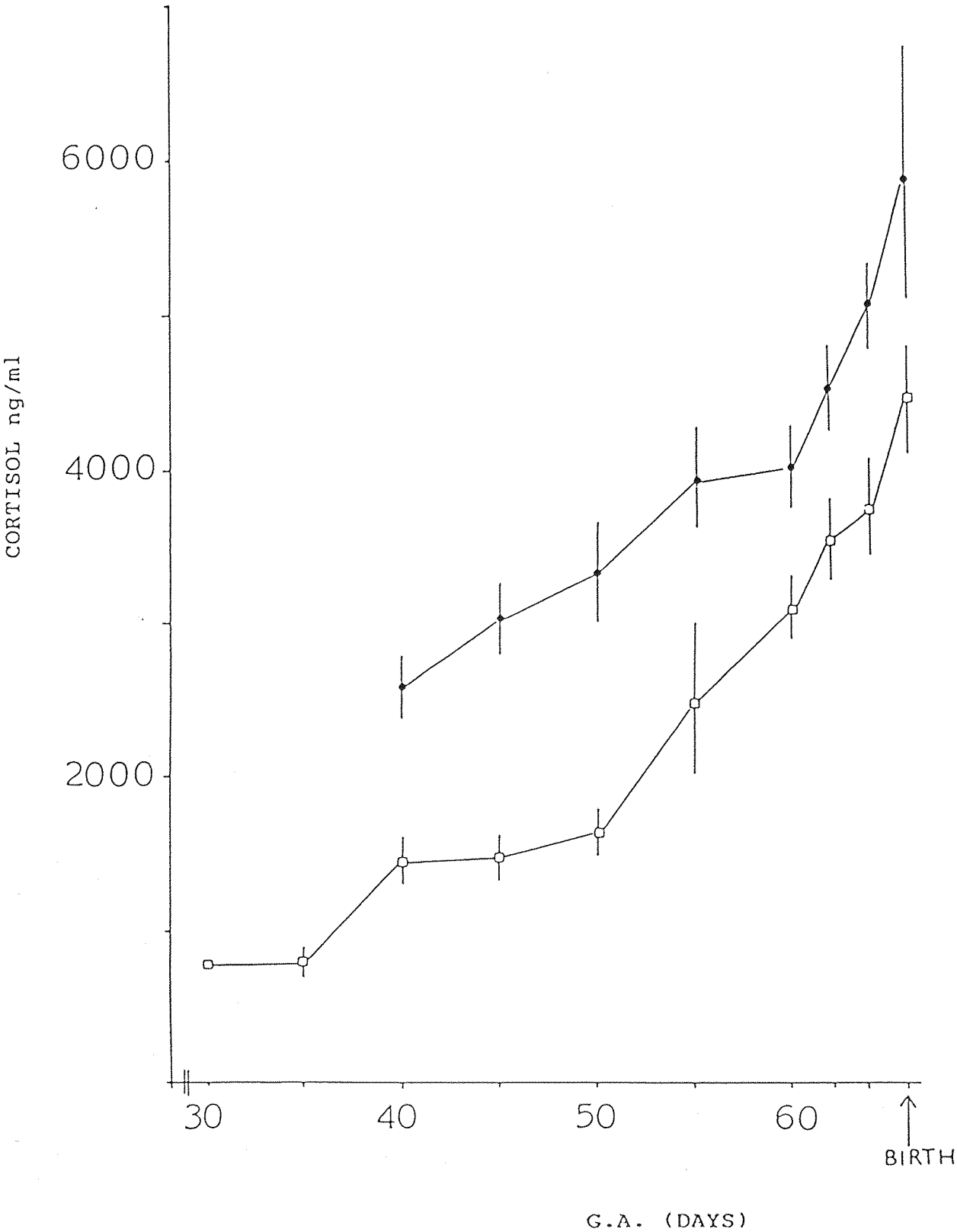


Figure R3-1 Changes in maternal plasma F concentration from 30 days of gestation to term :- after anaesthesia (●), without anaesthesia (□).

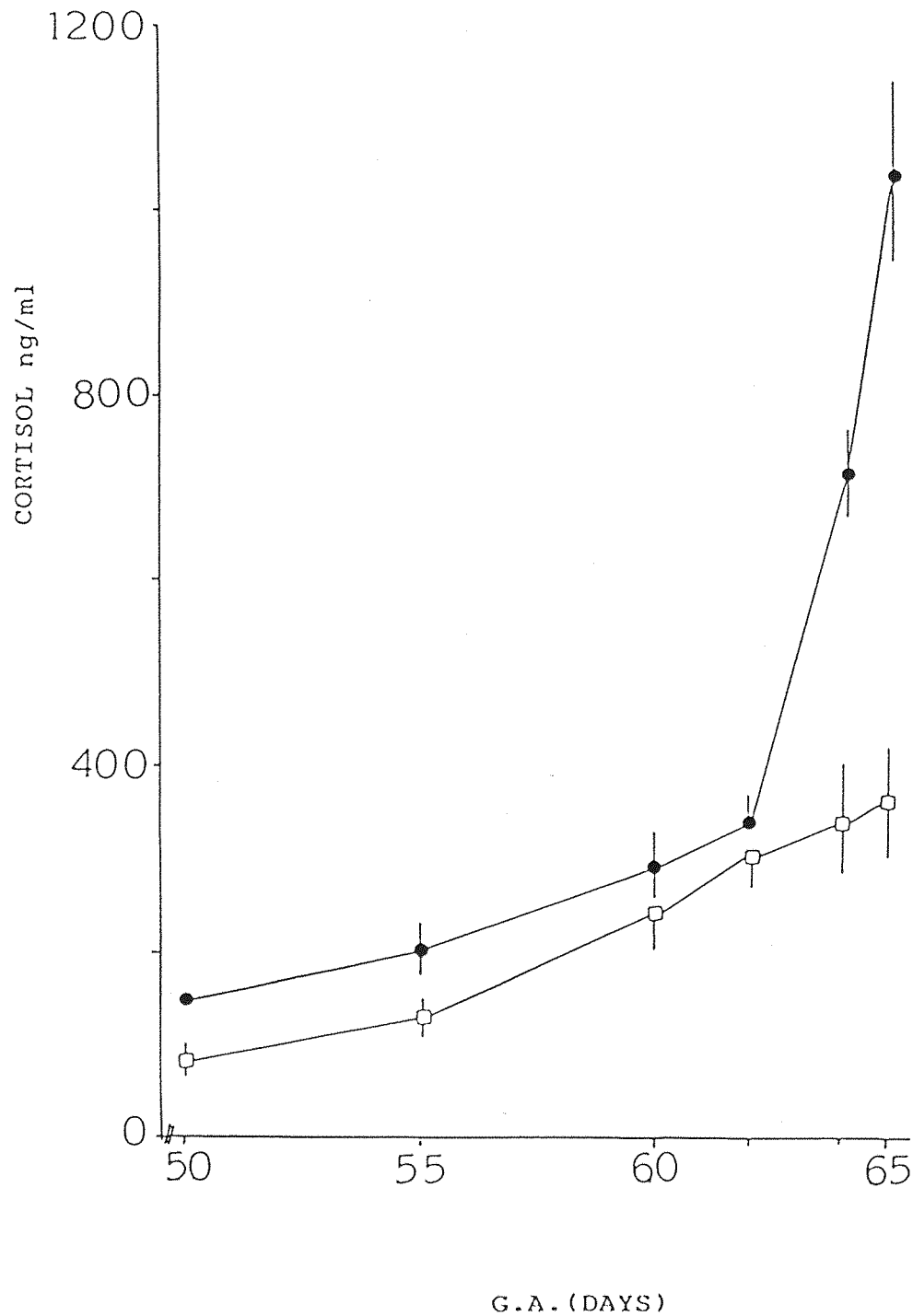


Figure R3-2 Changes in fetal plasma F concentration from 50 days of gestation to term :- after anaesthesia (●), without anaesthesia (□).

Postnatal Cortisol Concentration

Figure R3-3 and Table R4-3 show the plasma cortisol concentration in newborn male and adult male guinea pigs. Mean plasma cortisol concentrations increase after birth and were significantly increased ($P < 0.05$) at 9 hours compared with values at 1 hour. After 9 hours (1740 ± 170 ng/ml) plasma cortisol concentrations decreased significantly ($P < 0.01$) to a value of 1060 ± 115 ng/ml at 24 hours. Cortisol concentrations fell further to 48 hours when the value was, in turn, significantly ($P < 0.01$) higher than the value on day three. The levels then remained relatively constant until day 6 after which they fell steadily to day 21 (353 ± 20 ng/ml). The cortisol concentration in male guinea pigs at day 21 was still greater than that measured in adult male animals. The cortisol concentration measured in adult non-pregnant females was greater than that measured in adult males (Table R4-3).

Changes in fetal adrenal gland cortisol concentration

Fetal adrenal cortisol concentrations (ng/100 mg gland) are shown in Figure R3-4, and Table R3-1. The concentration of cortisol showed two peaks between day 35 and birth. In fetuses at day 35 the mean concentration was 1820 ± 352 ng/100 mg. This high concentration was followed by a sharp decline to 40 days and a further fall was observed at 45 and 50 days by which time the value was 612 ± 72 ng/100 mg. After this the gland cortisol concentration increased at 55 days and reached a second peak of 1470 ± 119 ng/100 mg at day 60. This rise coincided with a sharp increase in plasma cortisol concentration (Fig. R3-2). This was followed by a sharp decrease in gland cortisol concentration between day 62 and term (Table R3-4). The mean value at term was 348 ± 15 ng/100 mg, about one quarter of that seen at day 60.

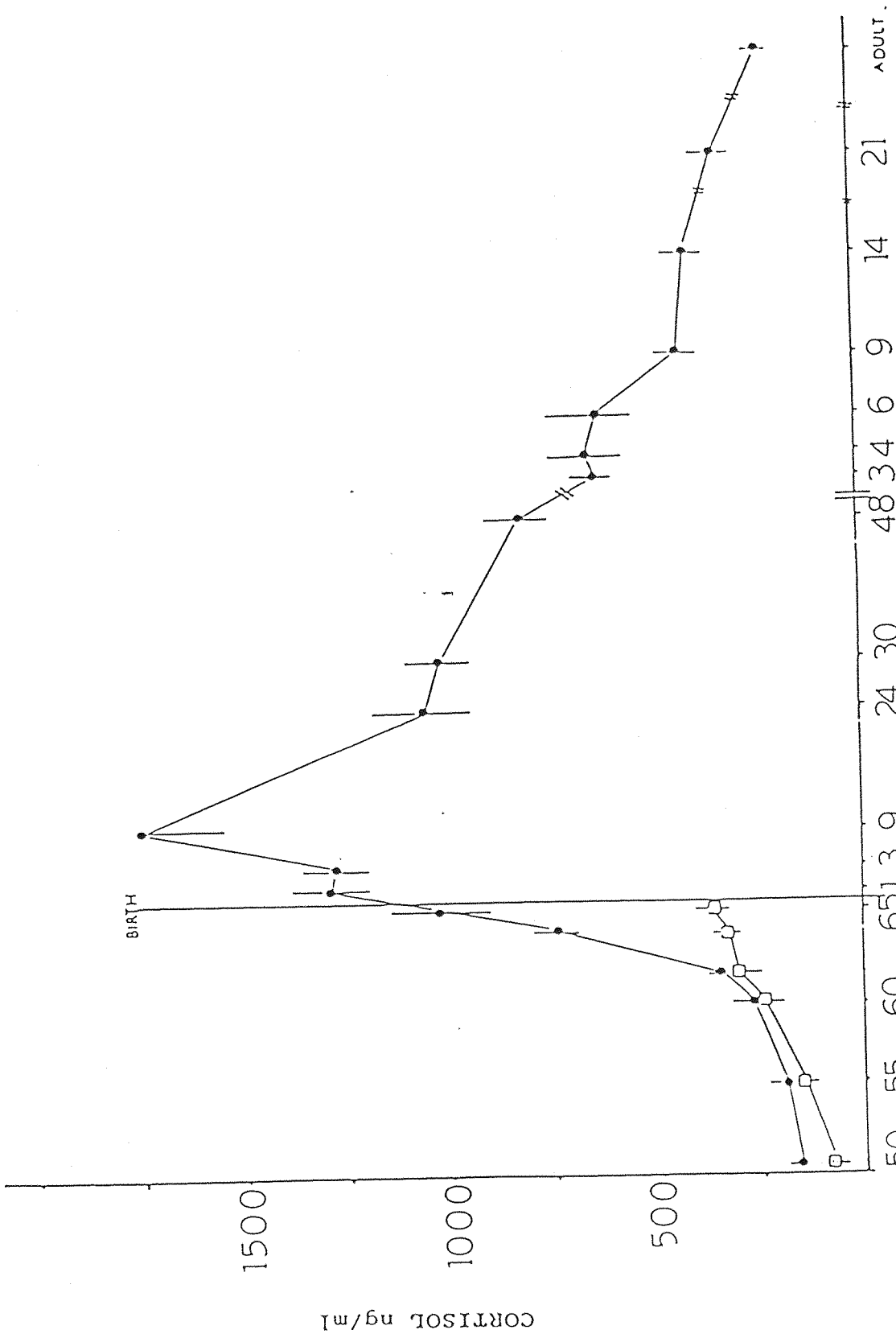


Figure R3-3 Changes in plasma F concentration in pre and post-partum guinea pigs :- after anaesthesia (●), without anaesthesia (□).

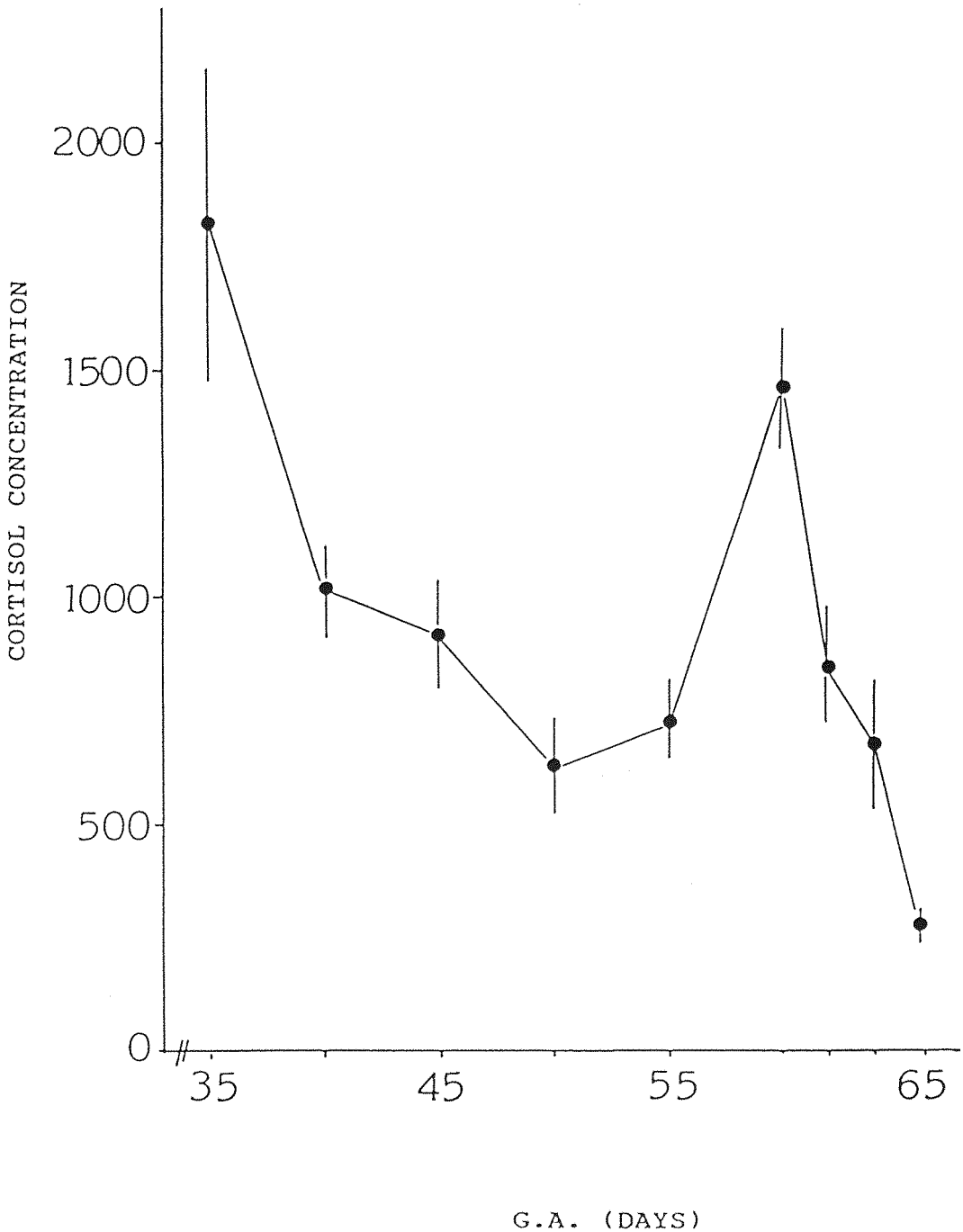


Figure R3-4 Adrenal F concentrations (ng/100mg adrenal tissue) of the fetal guinea pig from 35 days of gestation to term.

Results Section 4 - Thyroid Hormones

Introduction

plasma T4 and T3 concentrations and the proportion of free thyroxine were measured in maternal and fetal guinea pigs from 40 days of gestation to term, using two groups of animals (i) guinea pigs sampled after anaesthesia with sodium pentobarbitone (Sagatal) and (ii) pregnant guinea pigs which were killed quickly by a blow to the head and sampled immediately afterwards. Although the late fetal and maternal cortisol concentrations differed between the two groups, as expected, in view of the comparitively slower secretion and metabolism of thyroid hormones, there were no significant differences in fetal or maternal thyroid hormone concentrations between the two groups at any age and the results have therefore been pooled.

Fetal plasma T4 and T3 and thyroid gland T4 concentrations and Percentage Free Thyroxine

Fetal T4

The concentrations of T4 in fetal plasma are low at 45 days (18.5 $\pm$ 2.4 ng/ml). The concentration had increased significantly ($P < 0.05$) by 50 days (27.6 $\pm$ 2 ng/ml). Concentrations then again rose significantly ($P < 0.0001$) to reach the highest measured fetal T4 concentration at 55 days (36.7 $\pm$ 1 ng/ml). At this time the T4 concentration in the fetus was significantly greater ($P < 0.0001$) than the corresponding maternal value (25.4 $\pm$ 1 ng/ml; Table R4-1). Measurements of fetal plasma T4 concentrations were greater than corresponding maternal concentrations from 50 days of gestation until term although the difference was only significant between days 50 and 62. After 55 days of gestation fetal plasma T4 concentrations fell to reach 34.9 $\pm$ 2 ng/ml at 60 days, 30.0 $\pm$ 2.3 ng/ml at 62 days and 25.0 $\pm$ 2.5 ng/ml at 64 days by which time they were not

Gestation age in days	T4 Mother	T4 Fetus	T3 Mother	T3 Fetus
40	16.7 ± 1.2 (6)		1.7 ± 0.3 (4)	0.6 ± 0.1 (2)
45	18.5 ± 1.3 (6)	18.5 ± 2.4 (3)	1.9 * ± 0.5 (4)	0.981 ± 0.1 (9)
50	19.4 ** ± 0.8 (7)	27.6 ± 2.0 (12)	2.7 *** ± 0.3 (5)	1.5 ± 0.1 (13)
55	25.4 **** ± 1.1 (9)	36.7 ± 1.0 (19)	2.4 ** ± 0.2 (4)	1.7 ± 0.1 (21)
60	21.8 *** ± 1.6 (8)	34.9 ± 2.0 (21)	2.1 ± 0.3 (5)	1.7 ± 0.1 (22)
62	20.3 * ± 1.4 (5)	30.0 ± 2.3 (9)	1.9 * ± 0.3 (6)	1.3 ± 0.1 (9)
64	22.1 ± 2.0 (5)	25.0 ± 2.5 (10)	1.8 ** ± 0.1 (3)	1.1 ± 0.1 (8)
65-67	23.0 ± 1.0 (5)	25.0 ± 1.3 (13)	1.8 ± 0.1 (3)	0.96 ± 0.1 (21)

Table R4-1. Plasma T4 and T3 concentration (ng/ml) in the mothers and their fetuses during gestation.

significantly different from maternal values (Table R4-1 and Fig. R4-1). The half-life of this fall in fetal plasma T4 from day 60 to day 67 was 12.2 hours.

Fetal T3

The fetal plasma T3 concentrations changed in a similar manner to fetal plasma T4 concentrations with the important difference that fetal T3 concentrations were significantly less than maternal T3 concentrations at all stages of gestation except at 60 days (Table R4-1). Fetal T3 levels increased from 0.6 $\pm$ 0.1 ng/ml at day 40 to 1.5 $\pm$ 0.1 ng/ml at day 50 of gestation. Peak concentrations of T3 (1.7$\pm$ 0.1 ng/ml) occurred on days 55 and 60 of gestation after which fetal T3 concentrations fell significantly ($P < 0.05$) to day 62 and fell significantly ($P < 0.001$) again by day 64 (1.1 $\pm$ 0.1 ng/ml). The lowest levels,(0.96 $\pm$ 0.1) were found on days 65 and 67 (Fig. R4-2). There were no significant changes in maternal plasma T3 concentrations throughout the last third of gestation (Table R4-1).

Thyroid Gland Hormone Content

Table R4-2 shows the changes in T4 content (ng T4/gland) and concentration (ng T4/mg tissue) of the fetal thyroid gland in the latter part of gestation. There was a sharp ($P < 0.05$) decrease in T4 concentration from 50 days to 55 days. At 55 days the mean value had dropped to 0.5 $\pm$ 0.1 ng/mg tissue which was significantly ($P < 0.001$) less than the value at 60 days. The T4 concentration continued to increase to 1.25 $\pm$ 0.03 ng/mg tissue at 62 days. Fetal thyroid gland T4 concentration then decreased significantly ($P < 0.01$) to 0.95 $\pm$ 0.05 at 64 days and 0.72 $\pm$ 0.01 at term.

Percentage Free T4

The percentage of free thyroxine (%FT4) in the fetal plasma at 50

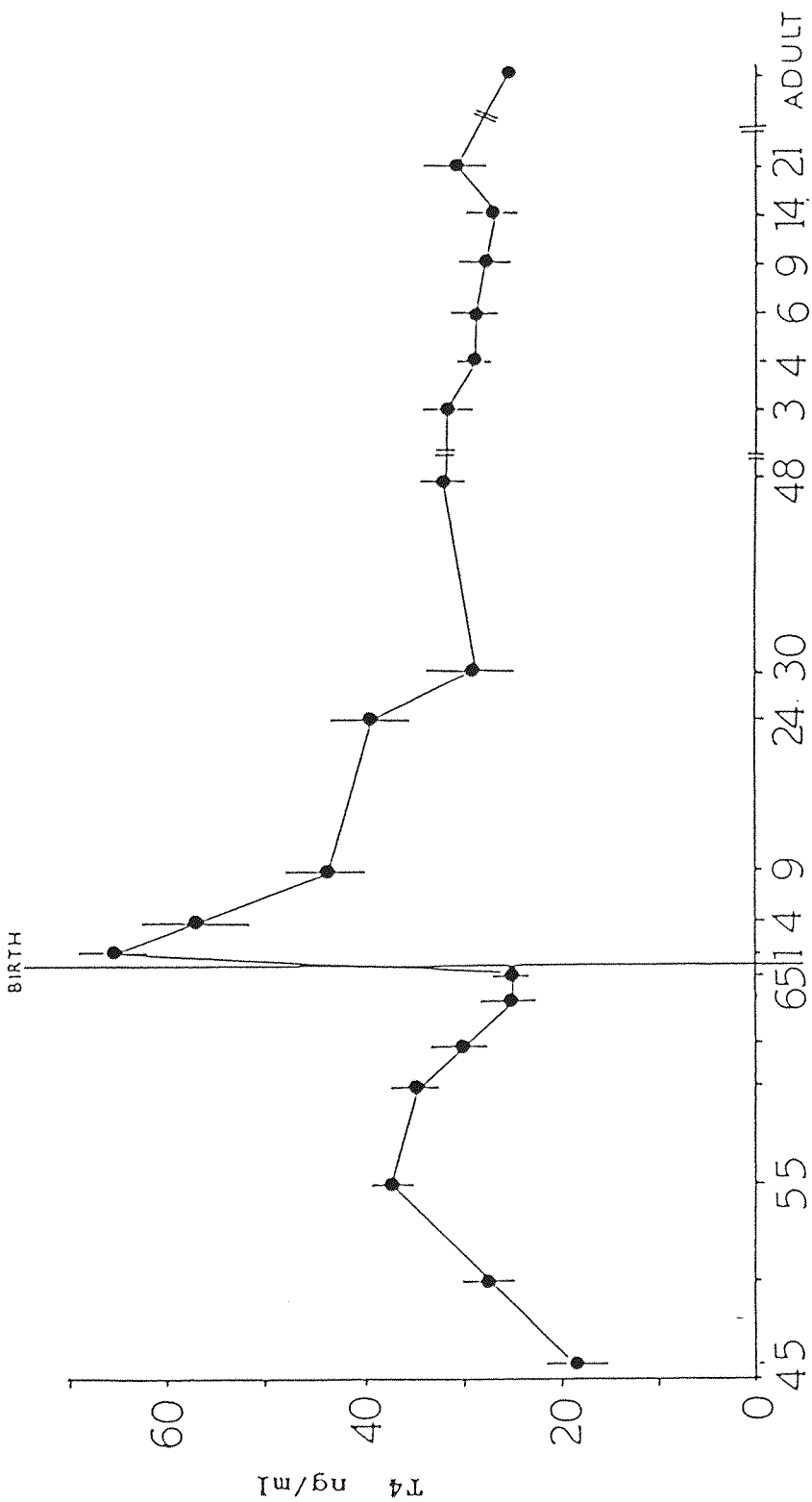


Figure R4-1 Changes in plasma T4 concentration in fetal and neonatal guinea pigs.

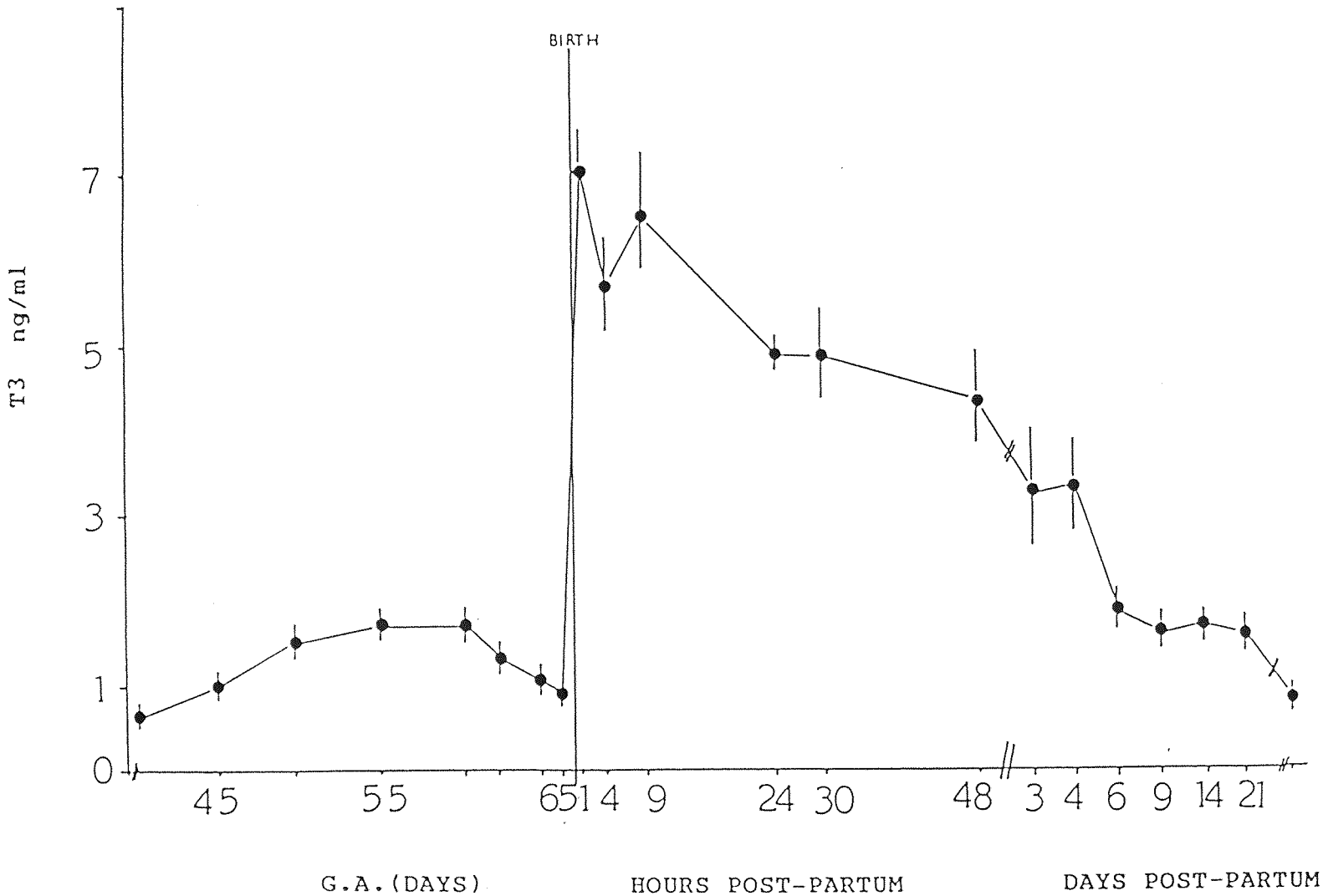


Figure R4-2 Changes in plasma T3 concentration in fetal and neonatal guinea pigs.

40	45	50	55	60	62	64	66
0.60	0.42	0.81 ± 0.02	0.52 ± 0.10	0.99 ± 0.10	1.25 ± 0.10	0.92 ± 0.05	0.72 ± 0.01
		(4)	(5)	(6)	(4)	(6)	(4)

Table R4-2. Fetal thyroid T4 concentration (ng/mg tissue) from 40 days to term.

days of gestation is 0.57 ± 0.08 and at 60 and 62 days the percentage has decreased but not significantly. There is a small increase in the proportion of unbound thyroxine at the end of gestation (65-67 days) when it measures 0.66 ± 0.186. Plasma %FT4 is high during the first hours after birth (0.57 ± 0.1) but falls quickly to reach values of 0.22 ± 0.03 by nine hours post partum which are significantly ($P < 0.001$) lower than the values measured at one hour (Figure R4-3).

Thyroid hormone concentrations in postnatal life

The mean values of plasma T4 and T3 concentrations for neonatal and young male guinea pigs aged from 1 hour to 48 hours and from 3 to 21 days are shown in Table R4:3 and Figure R4-1 & R4-2. There was a sharp rise in T4 levels during the first hour after birth to reach a value of 65.1 ± 3 ng/ml. By nine hours T4 levels had declined significantly ($P < 0.001$) to 44 ± 3 ng/ml. From 9 hours to 24 hours the T4 level declined to 38.5 ± 3 ng/ml and by day 3 it had reached 32 ng/ml, less than half the level at 1 hour. The half-life of the fall in plasma T4 concentration between 1 and 30 hours after birth was 27.7 hours which compares with the plasma T4 half-life of 23.4 ± 2.44 hours measured in the adult. This suggests that after one hour post partum neonatal thyroxine secretion is completely suppressed. No significant changes in T4 concentration were found between 4 and 21 days of postnatal life. At 21 days the plasma T4 concentration was 30.4 ± 2 ng/ml still significantly greater than that measured in adult male or non-pregnant female guinea pigs (Table R4-3).

Plasma T3 concentrations are elevated immediately after birth to levels eight to ten times those seen in the adult. Four phases can be seen in the changes in plasma T3 concentration from birth to 21 days.
(i) Concentrations were highest 1 to 9 hours after birth and had fallen significantly ($P < 0.0001$) by 24 hours to 4.8 ± 0.1 ng/ml.
(ii) From 24 to 48 hours the mean T3 level remained constant at about 4 ng/ml.

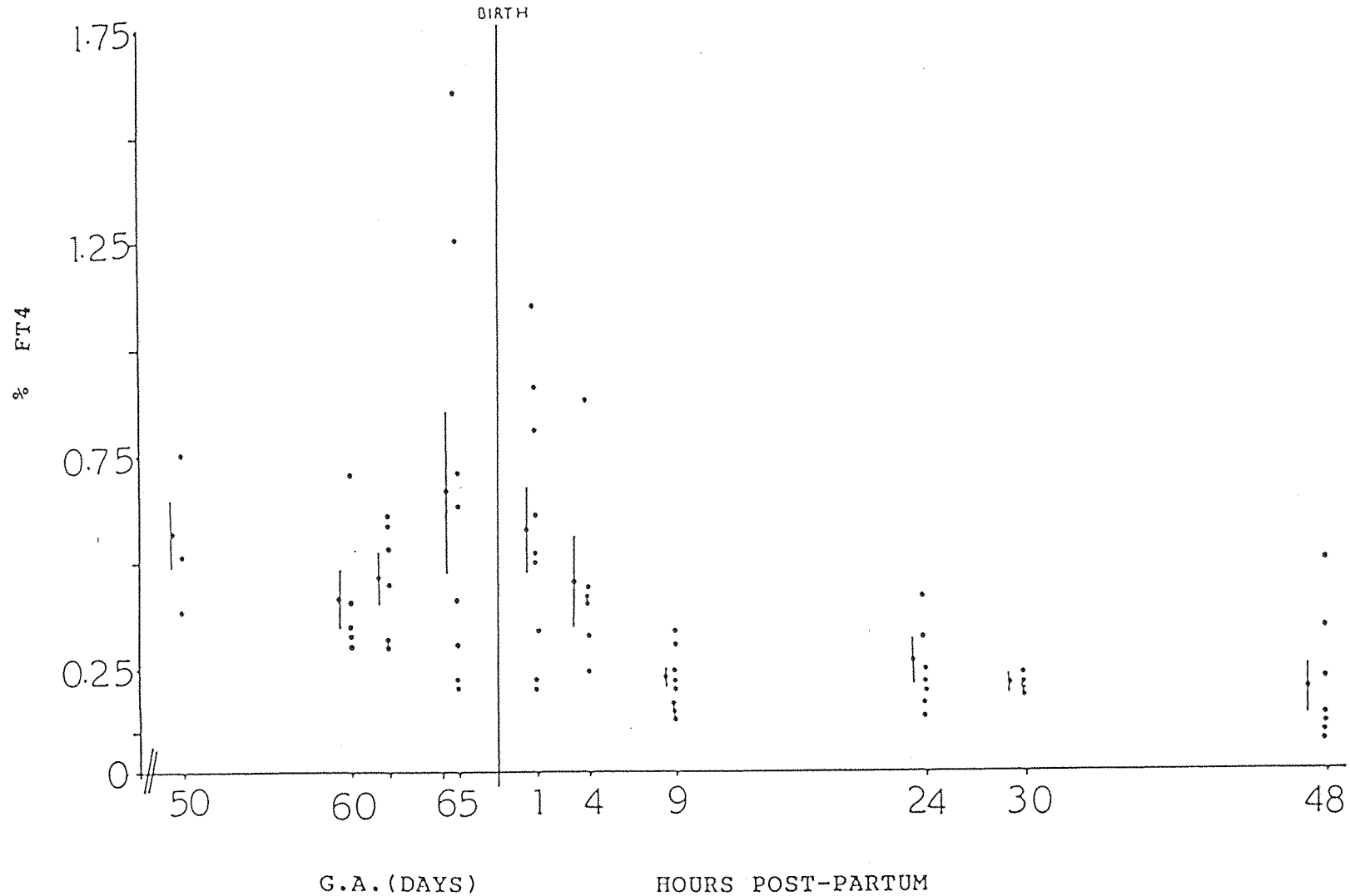


Figure R4-3 Changes in the plasma % FT4 of fetal and neonatal guinea pigs from 50 days of gestation to 48 hours after birth.

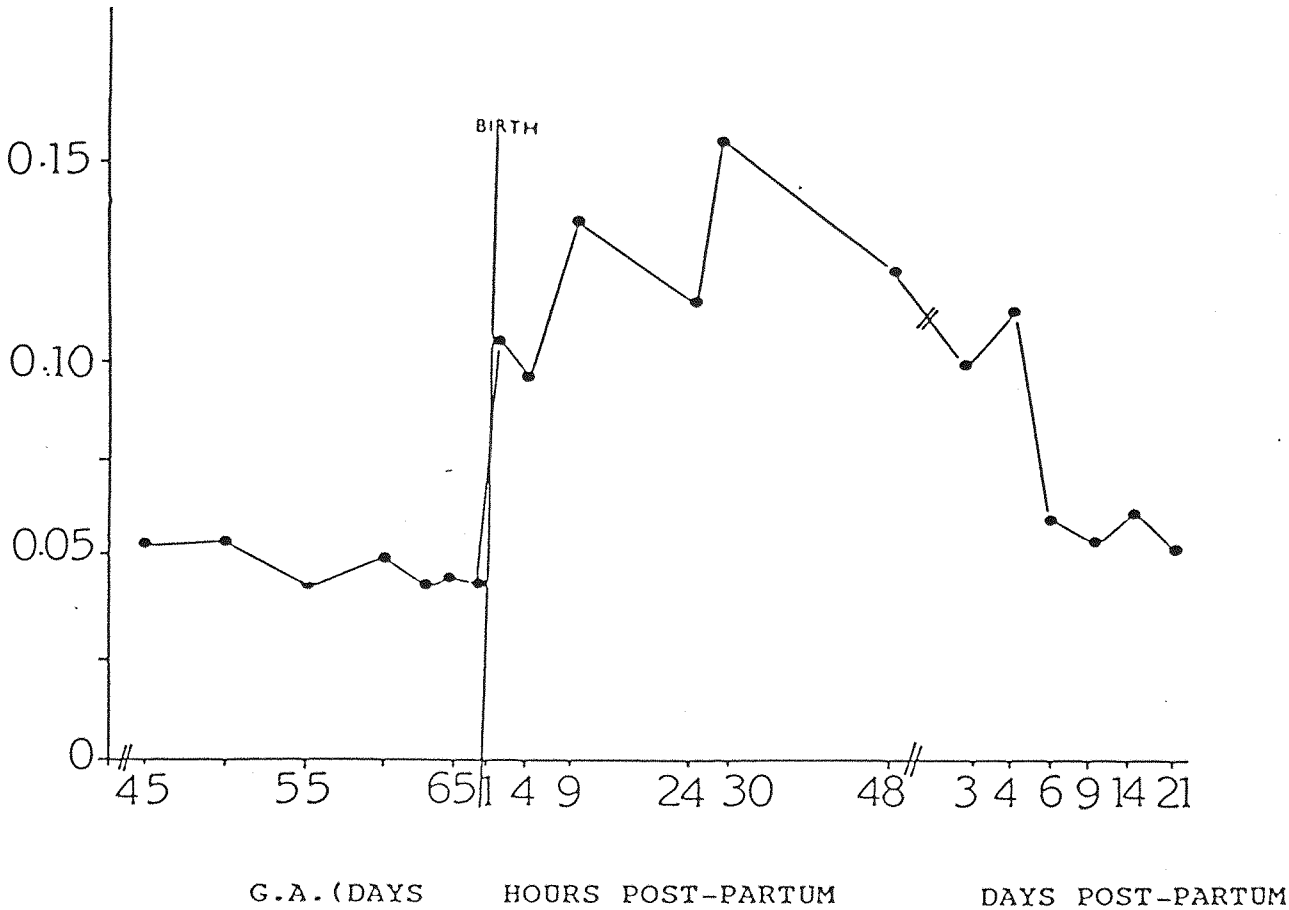


Figure R4-4 Changes in the plasma T_3/T_4 ratio in fetal and neonatal guinea pigs.

Age in	Cortisol	Thyroxine	Triiodothyronine
Hours			
1	1300.0 ± 82.0 (8)	65.0 ± 3.0 (17)	7.0 ± 0.5 (16)
4	1280.0 ± 80.0 (9)	57.7 ± 5.0 (12)	5.6 ± 0.5 (5)
9	1740.0 ± 170.0 (4)	44.0 ± 3.0 (14)	6.5 ± 0.7 (4)
24	1060.0 ± 115.0 (10)	38.5 ± 3.0 (5)	4.8 ± 0.1 (6)
30	1192.0 ± 142.0 (6)	28.5 ± 4.0 (4)	4.8 ± 0.4 (7)
48	840.0 ± 40.0 (8)	31.9 ± 2.0 (8)	4.3 ± 0.4 (4)
Age in Days			
3	656.0 ± 39.0 (6)	31.8 ± 2.4 (10)	3.2 ± 0.4 (8)
4	680.0 ± 100.0 (4)	29.2 ± 1.4 (15)	3.3 ± 0.5 (6)
6	628.0 ± 44.0 (11)	29.2 ± 1.2 (11)	1.8 ± 0.1 (6)
9	430.0 ± 27.0 (6)	27.6 ± 2.0 (9)	1.6 ± 0.1 (5)
14	423.0 ± 26.0 (15)	26.6 ± 1.2 (16)	1.7 ± 0.1 (6)
21	353.0 ± 20.0 (16)	30.4 ± 2.0 (13)	1.6 ± 0.1 (8)
Adult			
Male	270.0 ± 10.0 (6)	25.5 ± 1.3 (7)	0.7 ± 0.1 (6)
Female	514.0 ± 12.0 (5)	25.5 ± 0.5 (9)	0.6 ± 0.1 (6)

Table R4-3. Plasma cortisol, T4 and T3 concentrations (ng/ml) in neonatal guinea pigs.

(iii) On days 3 and 4 of postanatal life T3 concentrations had fallen to about 3 ng/ml.
(iv) By day 6 levels had fallen to 1.8 $\pm$ 0.1 ng/ml and remained at about this value until day 21 when the T3 (Fig. R4-2) concentration was still significantly greater (about two fold) than those seen in adult males or non pregnant females.

The T3/T4 plasma concentration ratio which is relatively constant between 45 and 67 days of gestation in the guinea pig fetus (ranging from 0.054 to 0.044) rises sharply immediately after birth to reach its highest level (0.168) at 30 hours of postnatal life falling again to 0.061 on day 6 (Figure R4-4).

Results Section 5 - Iodide Uptake

Introduction

Measurement of the radioactivity of fetal thyroid glands following administration of radioiodine to pregnant guinea pigs at early stages of gestation was used to determine the time in development when the fetal thyroid begins to function.

Experimental Procedure

Six pregnant guinea pigs were used in this experiment from the breeding colony. Pregnancies were dated by limiting the mating time and examining for vaginal plugs. At various stages of gestation pregnant females were injected I.P. with 50 µCi [^{125}I] solution. Solutions were made up by making 50 µCi [^{125}I] up to one ml with saline. The radioiodide injection was given 20 hours before sampling began. Fetuses used in this study were obtained by caesarian section at 30, 33 and 35 days following conception. The number of animals in each group is shown in Table R5-1. The pregnant guinea pigs were anaesthetised by I.P. injection of sodium pentobarbitone (40 mg/kg) and samples of maternal blood were obtained by cardiac puncture. The abdomen was opened in the mid-line, the uterine wall over each fetus was incised, the fetal membranes were exposed and a sample of amniotic fluid withdrawn by syringe and needle for measurement of amniotic fluid radioactivity. The fetuses were removed and weighed following separation from the placentae.

After maternal blood and amniotic fluids were sampled the mother was killed. Radioactivity in these fluids as well as in the maternal thyroid, placentae, fetal neck, muscles and liver was measured. These tissues were removed from mothers and their fetuses weighed and the radioactivity counted in a Clinigamma counter (LKB). The small size and lack of distinguishing colour of the thyroid gland in the young fetuses made it impractical to remove the thyroid alone and

therefore to avoid the loss of radioactivity the fetal neck was dissected out along with attached material and counted. It was possible to locate the thyroid within this tissue by repeatedly dividing and counting the material.

Calculation of radioiodide activity

50 µCi of [^{125}I] was diluted to 1 ml with saline. Pregnant guinea pigs received 0.9 ml and 0.1 ml was diluted to 50 ml with distilled water and used as the appropriate injection standard. The injection standards were counted at the same time as the corresponding tissues and fluids.

Total [^{125}I] counts in 50 µCi

= No. counts in 1 ml of inj. sol x 50 x 9

The iodide uptake of the fluids and tissues was expressed as tissue counts per gram or ml and calculated as a fraction of the total injected counts.

Results

The fetal guinea pig begins to concentrate iodide between the 34 and 35 days of gestation (Fig. R5-1 and Table R5-1). In the fetuses from mothers injected on the 34th day the percentage of [^{125}I] in the fetal thyroid material was 13 times more than the percentage in fetal liver on the same day of gestation and 15 times more than in the fetal thyroid material on the 29/30 day. There was a significant ($P < 0.001$) increase in the fetal thyroid material iodide uptake at 34/35 days compared with 32/33 days.

The percentage of the radioiodide accumulated by the maternal thyroid and plasma 20 hours after administration of the radioiodide varied between individuals at the same day of gestation. Generally the percentage decreased as gestation proceeded. During these five

days accumulation of iodide in the placental tissue was significantly ($P < 0.001$) greater at 32/33 days (group 2) than at either 29/30 days (group 1) or at 34/35 days (group 3). There will be competition between the maternal thyroid, the placentae and the fetal thyroid for the uptake of radioiodide. The fall in maternal thyroid and placental iodide uptake at 34/35 days coincided with the increase in fetal thyroid iodide uptake seen at this age.

The percentage of injected [^{125}I] contained in fetal liver, muscle and amniotic fluid varied only slightly from animal to animal. There was no change in the iodide concentratoon in these tissues with gestational age (Fig. R5-1).

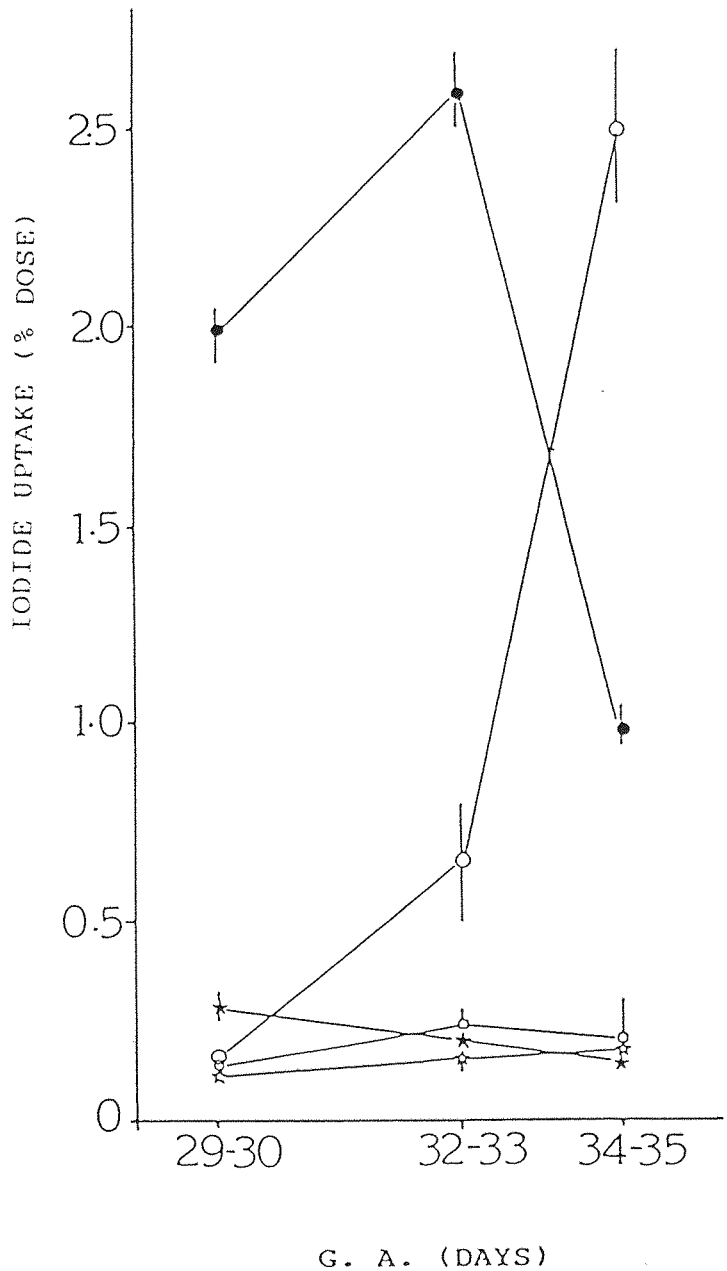


Figure RS-1 Uptake of ^{123}I by the placenta (●) and fetal tissue [Neck (○), Muscle (□), Amniotic fluid (★) and Liver(☆).] 20 hours after adminstration of 50 µCi ^{123}I to pregnant guinea pigs.

Gestation Days	Fetal Tissues				
	Neck	Muscles	Liver	Amniotic Fluid	Placentae
29-30 (6)	0.15 ± 0.02	0.13 ± 0.02	0.10 ± 0.01	0.27 ± 0.04	1.98 ± 0.07
32-33 (7)	0.65 ± 0.15	0.23 ± 0.04	0.15 ± 0.01	0.18 ± 0.01	2.60 ± 0.08
34-35 (6)	2.50 ± 0.22	0.20 ± 0.09	0.14 ± 0.02	0.13 ± 0.04	0.97 ± 0.03

Table R5-1. The radioiodide uptake by the fetal thyroid and other fetal tissues (expressed as % injected dose per g or per ml.

Results Section 6 - Plasma Thyroxine Kinetics

Introduction

In order to better understand the basis of the changes in thyroid hormone concentration in the newborn animals it was necessary to determine the kinetics of thyroid hormone in the adult guinea pig since there are no available data in the literature.

Determination of Plasma Thyroxine Kinetics

Thyroxine kinetics were investigated in normal adult male guinea pigs weighing 750-1050 g. Under 2 % Halothane/ oxygen/ nitrous oxide anaesthesia a cannula was inserted into the left jugular vein and extened outside through an incision at the back of the neck. A period of 60 hours was allowed for recovery from this operation and then a 2ml blood sample was taken for the measurement of plasma T4 concentration. After this 6 to 10 µCi of ^{125}I labelled T4 (according to body weight) was diluted in 0.5 ml of physiological saline and homologous plasma and injected into the jugular cannula. Sodium thiocyanate (100 mg) was administered immediately before and 48 hours after the radioactive thyroxine to prevent recirculation of the ^{125}I. Plasma samples for measurement of radioactivity were taken twice daily from 24 to 96 hours after the ^{125}I T4 injection and treated as for the free T4 assay to separate the T4 from free iodide.

Calculation

The thyroxine half-life, volume of distribution, metabolic clearance rate and secretion rate were calculated as follows.

An aliquot of each injection solution was diluted and counted to determine the total injected counts for each animal. The theoretical initial concentration of the radioactive thyroxine was determined by extrapolating the semi-logarithmic lot of the plasma counts against time to zero time.

[1] Volume of Distribution (VD) = $\frac{\text{Counts Injected}}{\text{Theoretical Initial Concentration}}$

[2] Thyroxine Pool = T4 concentration * VD

[3] $C_t = C_0 * e^{-kt}$

Thus k = fractional turnover rate = $\frac{\log_e 2}{\text{half-life}}$

[4] Thus :-

T4 secretion rate = VD * T4 concentration * k

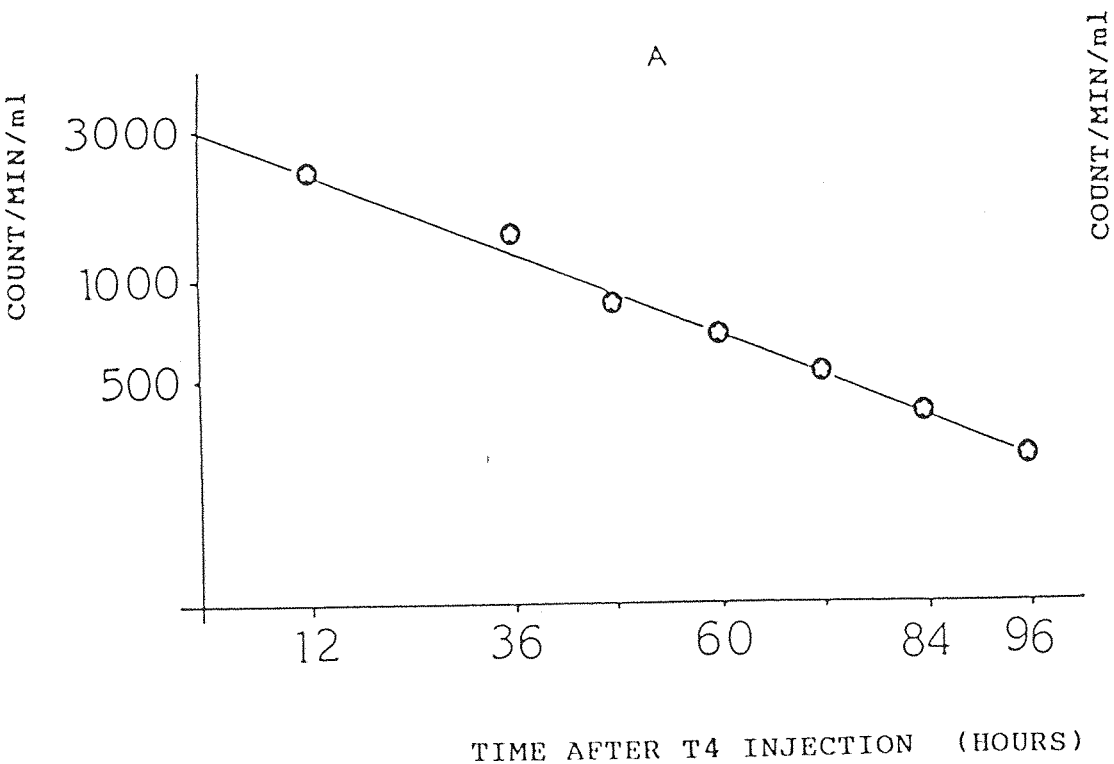
Results

Figures R6:1,2,3 and 4 show semilogarithmic plots of the disappearance of ^{125} I T4 from the blood. Table R6:1 shows the values obtained for the various parameters of thyroxine kinetics in the guinea pig.

Number of Animal	Animal Body Weight(gm)	VD mls	$T_{1/2}$ Hours	T4 Conc. ng/ml	Thyroxine Pool/ ng
1	950	275.40	28.64	29	7986.60
2	860	232.10	23.31	32	7426.23
3	1170	130.33	16.92	28	3649.24
4	1050	151.10	24.81	28	4230.42
Mean	1007.5	197.23	23.42	29.25	5823.12
± SEM	± 66.63	± 34.07	± 2.44	± 0.95	± 1099.74

T4 secretion ug/kg/day	MCR ml/day	K day^{-1}
4.85	159	0.579
6.1	165	0.711
3.06	128	0.979
2.69	101	0.668
4.17 ± 0.80	138.25 ± 14.83	0.73 ± 0.09

Table R6-1. The volume of distribution, T4 half life ($t_{1/2}$), plasma T4 concentration, thyroxine secretion rate, metabolic clearance rate (MCR), thyroxine pool and fractional turnover rate (K) in four adult male guinea pigs.



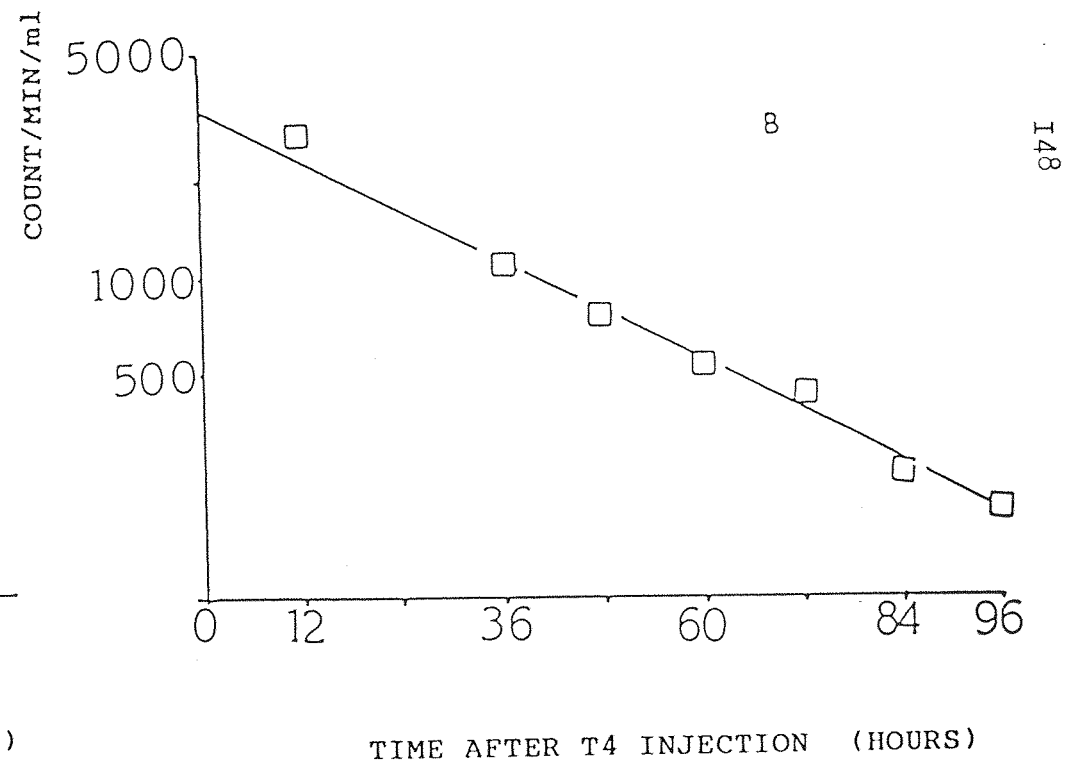
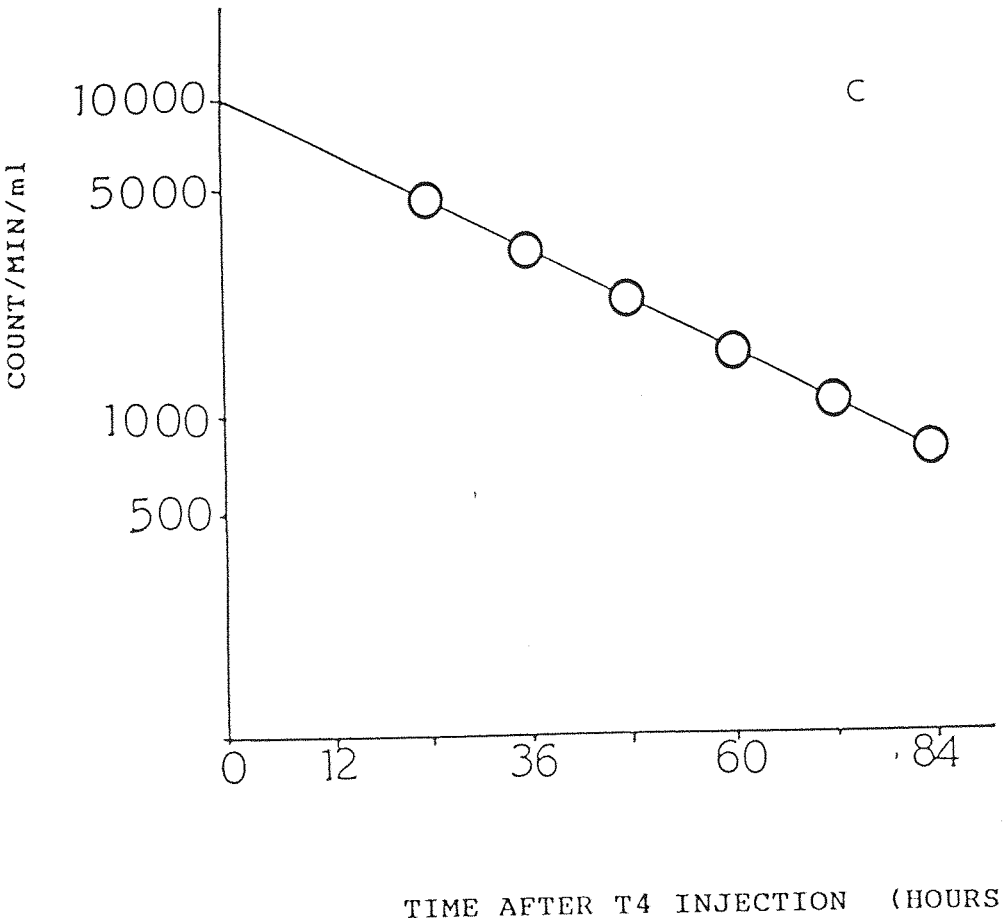


Figure R6-1 (a-d) The disappearance of ^{125}I labelled T4 from the plasma of 4 adult male guinea pigs.



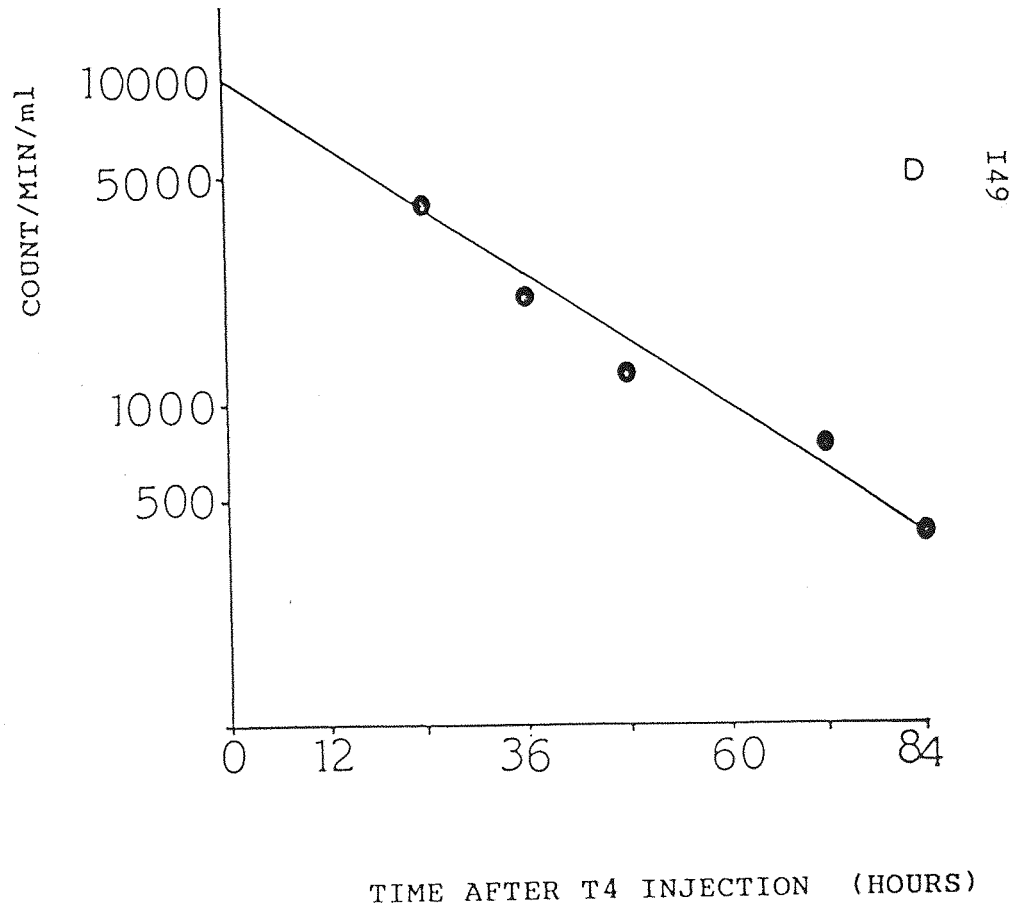


Figure R6-1 (a-d) The disappearance of ^{125}I labelled T4 from the plasma of 4 adult male guinea pigs.

Results Section 7 - Dexamethasone

Introduction

In pregnant guinea pigs and their fetuses plasma T4 and T3 levels were decreased during the last few days of gestation at a time when fetal and maternal plasma cortisol concentrations were high. To investigate the cause of this decrease further the synthetic glucocorticoid dexamethasone was administered daily to pregnant guinea pigs on 55 to 60 days of gestation to produce an increase in plasma glucocorticoid activity which resembled that normally seen in late gestation. The effects of treatment were observed on maternal plasma concentrations of cortisol, thyroxine and triiodothyronine and fetal plasma and tissue concentration of cortisol, thyroxine, triiodothyronine and % FT4. The fetal body weight, and thyroid, adrenal and pituitary weights as well as the placental weights were recorded.

Materials and Methods

After controlled mating female guinea pigs from the breeding colony were maintained in the routine environment of the colony. Dexamethasone (Decadron, MSD Ltd) was administered in a dose of 10 mg subcutaneously daily from day 55 to day 60 of gestation. On gestation day 60 the last injection was given six hours before sampling began. The guinea pigs were killed by a blow to the neck and blood samples were quickly collected into EDTA tubes. The fetuses were quickly bled by cardiac puncture. Fetal carcasses and placentae were removed, blotted on filter paper and weighed. Fetal thyroid, adrenal and pituitary glands were removed, cleaned of surrounding connective tissue and weighed on an Oertling balance. The adrenal and thyroid glands were frozen at -20°C until assay.

Each adrenal gland was homogenised in 0.5 ml normal saline and 0.1 ml of this homogenate was extracted with 2 ml of ethanol, centrifuged and the supernatant evaporated and assayed. Thyroid glands were

thawed and frozen 4 to 6 times just before homogenising in 3 ml of n-butyl alcohol. The homogentae was centrifuged for 10 minutes at 2000 rpm and then 1 ml of the supernatant was evaporated down for thyroxine assay .

Results

The absolute weights of the left adrenal and thyroid glands in the dexamethasone treated fetuses was significantly ($P < 0.001$) less than those of untreated fetuses. The relative weights (mg/100g body weight) were also siginificantly less ($P < 0.05$) in treated fetuses. Fetal body weights of dexamethasone treated fetuses were significantly ($P < 0.01$) less than those of untreated fetuses. No significant differences were found in the pituitary and placental absolute or relative weights (Table R7-1). There were no signs of abnormal development.

Changes in cortisol concentration

The cortisol concentration of the adrenal gland (ng/100 mg adrenal tissue) was significantly ($P < 0.01$) less in fetuses exposed to dexamethasone than those of untreated fetuses of the same age. There was no apparent effect of dexamethasone administration on maternal or fetal plasma cortisol concentration (Table R7-2).

Changes in thyroid hormone concentration

Thyroid hormone levels in the maternal and fetal plasma were less in dexamethasone treated animals. Administration of dexamethasone resulted in a significant decrease ($P < 0.0001$) in the fetal plasma thyroxine concentration and %FT4 (Table R7-3). A significant ($P < 0.001$) increase in the thyroxine concentration (ng/mg tissue) was observed in the thyroid glands of dexamethasone treated fetuses compared with normal fetuses (Table R7-3). The dexamethasone treatment also caused a significant ($P < 0.05$) decrease in the maternal plasma T4 and T3 concentrations of the 60 day pregnant guinea

pigs compared with untreated pregnant animals. No significant difference was found in the plasma T3/T4 ratio between the two groups. (Table R7-4).

The T3 concentration in the fetal plasma was significantly ($P < 0.05$) lower in the dexamethasone treated fetuses compared with untreated animals. The plasma T3/T4 ratio in the dexamethasone treated fetuses was significantly ($P < 0.001$) higher than that of normal 60 day old fetuses (Table R7-4).

	Body weight	Placental Wt		Thyroid Wt	
		mg	gm/100gm body weight	mg	mg/100gm body weight
Normal	57.0 ± 0.9 (26) **	4.2 ± 0.2 (12)	7.9 ± 0.4	21.2 ± 0.6 (26) ***	37.8 ± 1.2 *
Dexa	52.0 ± 1.8 (17)	3.8 ± 0.3 (17)	7.2 ± 0.3	17.5 ± 0.8 (17)	33.8 ± 0.9

	Adrenal WT		Pituitary WT	
	mg	mg/100mg body weight	mg	mg/100gmbody weight
	9.9 ± 0.5 ***	17.2 ± 0.9 *	2.4 ± 0.2	4.3 ± 0.3
	7.4 ± 0.3	14.3 ± 0.6	2.0 ± 0.2	3.9 ± 0.5

Table R7-1. Comparison between the relative and absolute weight of thyroid, adrenal and pituitary glands of dexamethsone treated and normal fetuses (mg/100gm body weight).

	Fetal Plasma ng/ml	Fetal gland ng/100mg tissue	Maternal plasma ng/ml
Normal	248.0 ± 23.6 (8)	1470 ± 119.0 (11) **	3187 ± 13.0 (4)
Dexa.	232.6 ± 16.0 (15)	821 ± 142.0 (17)	2705 ± 394.0 (4)

Table R7-2. Changes in the maternal plasma, fetal plasma and adrenal gland cortisol concentration.

	Maternal T4	Fetal T4	T4/Gland ng/mg Tisse	Fetal T4 %
Normal	21.80 ± 1.60 (8) *	34.90 ±2.00 (16) ***	0.99 ± 0.10 (6) ****	0.41 ± 0.07 (5) ****
Dexa	12.90 ± 2.40 (4)	17.5 ± 30.00 (13)	1.69 ± 0.10 (13)	0.16 ± 0.04 (6)

Table R7-3. Maternal and fetal plasma T4 concentration (ng/ml) and fetal % FT4.

	Fetal	Maternal	Maternal T3/T4 Ratio	Fetal T3/T4 Ratio
Normal	1.7 ± 0.1 (16)	2.5 ± 0.1 (4)	0.12 ± 0.01 (5)	0.05 ± 0.01 (19)
	*	*		***
Dexa	1.38 ± 0.06 (14)	1.92 ± 0.20 (4)	0.16 ± 0.03 (4)	0.12 ± 0.02 (14)

Table R7-4. Fetal and maternal plasma T3 concentration (ng/ml).

Results Section 8 - PTU Treatment

Introduction

The operation of the pituitary thyroid feedback mechanism is seen clearly in experiments which interfere with hormone synthesis either by depriving the animal of dietary iodide, by inhibiting thyroid transport of iodide with perchlorate or by preventing the iodination of protein using one of the drugs of the thiouracil group. All of these treatments result in a decline in circulating thyroid hormones which stimulates the pituitary to release more TSH and hence leads to thyroid enlargement. It was hoped that by administering antithyroid compounds to pregnant guinea pigs it would be possible to gain an indication of the level of activity of the fetal thyroid gland at various stages in gestation. It was also intended to determine the histological changes of the fetal thyroid gland during PTU treatment and examine the effects on fetal ossification and growth.

Materials and Methods

Male and Female guinea pigs used in this experiment were fed propylthiouracil (PTU, Sigma). PTU was mixed with food 0.1% by weight (D'angelo, 1966a). Experimental animals were grouped as follows.

1. Three adult males fed PTU for 50 days

2. Five pregnant guinea pigs 10-14 days of pregnancy were treated with PTU until spontaneous delivery occurred.

3. Six females were fed PTU on the day of conception and PTU treatment was continued until the pregnant guinea pigs were used as follows :- One guinea pig was sampled on day 30 and the second

on day 35. The third and fourth pregnant guinea pigs were sampled on day 50 and the last two on day 60.

4. The experiment included 23 fetuses and 19 neonates and these were distributed according to the following age groups 30 day, 4; 35 day, 5; 50 day, 9; 60 day, 5 and neonatal 19.

Blood was obtained from PTU treated males, mothers and their fetuses and newborn by cardiac puncture under sodium pentobarbitone anaesthesia. The dose of anaesthetic was divided as before. After samples of cardiac blood had been taken from the pregnant guinea pigs a mid-line abdominal incision was made and each fetus in turn exposed, cleared from the surrounding membranes and blood was collected by puncturing the heart. Blood samples were centrifuged in EDTA tubes and the plasma was frozen at -20°C until assay.

Fetuses and neonates were weighed and photgraphed. In order to provide an estimate of osseous development X-Ray photographs of whole body, fore and hind limbs of the neonates were taken. Thyroids, adrenals and pituitaries were removed from fetuses and neonates, trimmed and weighed on an Oertling balance. Skin and long bone samples were taken from the neonates. All material was prepared for histological examination.

Radiographs of the fore, hind limbs and whole body of the PTU treated and normal neonatal guinea pigs were taken using a cardioversion X-Ray machine (G.E.C., Watson) and Kodak film. The length of the long bones were measured using callipers. Films were developed using Kodak DX 80 developer (diluted 1 in 4) for 3 minutes after which the film was fixed and then washed under tap water for at least one hour before air drying.

Results

1. Fetal Weights

Changes in body, placenta, thyroid, adrenal and pituitary weights in the fetus and newborn fed PTU are shown in Table R8-1.

A. 30 days:- No significant differences were found in the fetal body weight and placental weights obtained from PTU fed or untreated mothers.

B. 35 days:- As at 30 days no differences were found in the body weights or in the left adrenal gland weights but the mean absolute placental weight was significantly greater ($P < 0.05$) in PTU treated animals compared with untreated animals. No significant difference was found in relative placental weight (placental weight/ fetal body weight) between the two groups.

C. 50 days:- At this stage of pregnancy the PTU treated fetuses had thin and hairless skin, internal organs and bones could clearly be seen (Fig. R8-1) The thyroid glands of the PTU treated fetuses had reached weights of 688 $\pm$ 50 mg about fifty times the weight of those of untreated fetuses. While the mean body weight of PTU treated fetuses was significantly ($P < 0.05$) lower than that of untreated fetuses. The mean absolute weight of the left adrenal gland was less ($P < 0.01$) than that of the untreated fetuses as was the mean relative weight ($P < 0.05$). No changes were observed in the relative or absolute weights of the placentae or pituitaries obtained from PTU and untreated fetuses(Table R8-1)

D. 60 days:- A sharp increase had occurred in the weight of the thyroid gland which had grown to each 2900 $\pm$ 142 mg compared with the mean weight of 21.2 $\pm$ 0.6 mg in untreated fetuses and more than four times the weight of the gland in 50 day PTU treated fetuses. The mean body weight of the treated fetuses was significantly ($P < 0.0001$) greater than those of untreated animals. Also the mean absolute and

Days of gestation		Body weight g	Placental g	Placental g/100g b wt	Thyroid mg	Thyroid %	Adrenal mg	Adrenal %	Pituitary mg	Pituitary %
30										
PTU Fetuses	(4)	1.61 ± 0.03	0.74 ± 0.03	45.96 ± 1.66						
Normal	(6)	1.45 ± 0.05	0.71 ± 0.05	48.96 ± 6.63						
35										
PTU Fetuses	(5)	5.42 ± 1.45	1.56 ± 0.15	28.78 ± 2.42	5.10 ± 0.46	94.09 ± 6.30	1.48 ± 0.14	27.30 ± 2.60	0.50 ± 0.04	9.22 ± 0.60
Normal	(11)	3.90 ± 1.73	1.20 ± 0.08 *	30.76 ± 0.83			1.23 ± 0.08	31.5 ± 1.40		
50										
PTU Fetuses	(9)	31.90 ± 0.90	3.23 ± 0.07	10.12 ± 0.30	688.11 ± 50.00	2157 ± 150.00	6.00 ± 0.33	18.80 ± 0.95	1.38 ± 0.16	4.32 ± 0.50
Normal	(10)	34.40 ± 0.95 *	3.44 ± 0.07	10.00 ± 0.33	13.80 ± 0.39	40.11 ± 1.11	7.60 ± 0.28 **	22.09 ± 0.97 *	1.63 ± 0.17	4.74 ± 0.66
60										
PTU Fetuses	(5)	70.70 ± 1.41	5.76 ± 0.20	8.14 ± 0.16	2900.0 ± 142.00	4101 ± 232.00	9.00 ± 0.47	12.70 ± 0.44	5.00 ± 0.23	7.07 ± 0.25
Normal	(26)	57.00 ± 0.93 ****	4.20 ± 0.12 ***	7.36 ± 0.26	21.20 ± o.60	37.20 ± 1.16	9.85 ± 0.47	17.28 ± 0.09 ****	2.42 ± 0.13 ****	4.24 ± 0.25 ****
Neonatal										
PTU	(19)	88.10 ± 4.25			4950 ± 829.00	5618.6 ± 700.00	9.87 ± 0.90	11.20 ± 0.07	7.80 ± 0.05	8.79 ± 0.07
Normal	(28)	83.70 ± 2.37			25.90 ± 1.30	30.94 ± 1.10	11.30 ± 0.64 *	14.50 ± 0.60 ***	3.68 ± 0.20 ****	4.42 ± 0.20 ****

Table R8-1. Effect of PTU on the fetal body weight and the absolute (mg) and relative (mg/100gm body weight) weight of the placenta and fetal thyroid, adrenal and pituitary.

relative weight of the pituitary gland was significantly ($P < 0.0001$) more than that in untreated fetuses. No significant changes were found in the absolute weights of the adrenal gland while the mean relative weight of the gland obtained from untreated fetuses was significantly greater ($P < 0.0001$) than the mean value of PTU fetuses. Placental mean absolute weight was significantly ($P < 0.001$) greater in the PTU treated fetuses than the mean value of untreated animals. No change in the mean relative weight was found (Table R8-1).

2. Newborn weights

Five pregnant guinea pigs were allowed to deliver naturally. In four animals the gestation was prolonged to 69-70 days compared with 66-68 days in normal animals. The fifth pregnant guinea pig bled vaginally at 63 days and delivered at 65 days. The guinea pigs gave birth to 22 newborn in five litters. The smallest litter of one individual was born alive whilst in the largest litter of eight animals 1 was mummified and born within its membranes apparently having died at about 50 days, 3 were born alive and died immediately afterwards and four survived. Two litters of four individuals were born alive and died immediately whilst in the last litter of 5 individuals 2 had obviosly died in utero at about 55 days and 3 were born alive and died immediately. From 22 animals 3 died in utero during gestation, 5 were born alive and were killed after sampling and 14 were born alive and died immediately after birth (Fig. R8-2)

Body weights of 19 newborn from goitrogen treated mothers ranged from 55 to 118 g (average 88.4 $\pm$ 4.3 g including thyroid weight and 83.2 $\pm$ 3.8 excluding thyroid weight, Table R8-2) while untreated mothers gave birth to newborn with body weights ranging from 62-107 g average 83.7 $\pm$ 2.4 g (Table R8-1). The mean weight of the thyroid glands from the 19 newborn PTU treated animals was 4940 $\pm$ 829 mg (ranging from 1420 mg to 17200 mg) 178 times the mean thyroid weight of normal newborn (25.9 $\pm$ 1.3 mg). The PTU thyroid gland capsule was thickened, the trachea, where it was surrounded by the two lobes

PTU Neonatal	Body weight g	Body weight -Thyroid wt	Thyroid weight mg	%
Stillborn (14)	92.11 ± 4.40	86.20 ± 3.80	5920.0 ± 1000.00*	6427 ± 800.00
Born live (5)	76.78 ± 9.53	74.60 ± 9.44 *	2190.0 ± 211.00 *	2852.3 ± 40.00

Adrenal weight mg	%	Pituitary weight mg	%
10.31 ± 1.20	11.19 ± 0.94	7.75 ± 0.25	8.41 ± 0.60
8.80 ± 0.66	11.46 ± 0.40	6.77 ± 0.36	8.81 ± 0.20

Table R8-2. The differences in the body weight (g) thyroid, adrenal and pituitary gland weights (mg) and relative weight (mg/100g fetal body weight). In stillborn and living offspring of PTU treated pregnant guinea pigs.

had a thinner wall and narrower lumen than in controls. The absolute mean weight of the left adrenal in the normal newborn was 11.3 ± 0.5 mg while the weight in the treated newborn was 9.9 ± 0.9 mg, significantly ($P < 0.05$) less. The relative adrenal weight was significantly ($P < 0.001$) higher in the normal than the treated newborn. The pituitary gland weights of the PTU treated newborn was 7.8 ± 0.05 mg which is more than twice that of normal newborn (3.7 ± 0.2 mg). The difference was significant ($P < 0.0001$). The relative pituitary weight was also significantly ($P < 0.0001$) greater in the PTU treated newborn.

It was found that the mean body weight of PTU treated newborn which died immediately after birth was 92.1 ± 4.4 g greater than that of the PTU treated animals which survived (76.8 ± 9.5g) however the difference was not significant. A significant difference was observed in the absolute and relative weights of the thyroid gland obtained from those animals which died after birth (5920 ± 1000 mg and 6200 ± 800 mg/100 g body weight) and those which survived birth (2190 ± 211 mg and 2900 ± 400 mg/100 g body weight). No differences were observed in the absolute and relative weights of the adrenal and pituitary glands (Table R8-2).

3. Adult weights

The absolute and relative weights of the thyroid and left adrenal gland were determined in :-

(i) Pregnant guinea pigs fed PTU and sampled after birth

(ii) Normal, non pregnant females

(iii) Normal pregnant females sampled just before term

The absolute and relative weights of the thyroid gland in group (i) were significantly ($P < 0.05$) higher than those of group (ii) and significantly ($P < 0.05$) higher than the absolute mean of group (iii)

Gestation age/ days	Body weight	Thyroid mg	Thyroid %	Adrenal mg	Adrenal %
50					
Normal (3)	778.0 ± 36.0	114.0 ± 6.5	14.8 ± 1.4	212.0 ± 25.2	27.1 ± 1.9
PTU (2)	715.0	135.0	19.6	335.0	47.0
60					
Normal (3)	776.7± 40.0	98.0 ± 9.2	12.7 ± 3.6	280 ± 28.4	36.0 ± 3.8
PTU (2)	800.0	120.0	15.1	330.0	41.4
After birth					
Normal Pregnant (4)	811.0 ± 34.0	112.3 ± 6.0	14.0 ± 1.0	290.3 ± 27.0	36.0 ± 4.4
PTU pregnant (5)	861.0 ± 38.0	146.0 ± 11.0	17.0 ± 1.0	424.0 ± 31.0	50.0 ± 4.0
Normal not Pregnant (6)	765.0 ± 35.0	102.0 ± 9.0	13.3 ± 1.0	196.0 ± 16.0	25.6 ± 3.0
Male					
Normal (6)	906.0 ± 51.0	110.3 ± 10.0	12.2 ± 1.0	288.0 ± 18.0	32.2 ± 2.0
PTU (3)	1006.0 ± 41.0	156.0 ± 20.0	15.5 ± 2.3	243.3 ± 9.0	24.3 ± 1.2

Table R8-3. Effect of PTU treatment on body and endocrine gland weight in the pregnant female guinea pig.

(Table R8-3). While the absolute and relative mean weights of group (i) adrenal gland were significantly ($P < 0.0001$) greater than that of group (ii) and significantly less ($P < 0.05$) than the absolute mean of group (iii).

In males no significant differences were observed in the mean weights of thyroid and adrenal glands between PTU and normal animals but the mean weight of the thyroid gland obtained from PTU treated males (156 $\pm$ 20 mg) was greater than those of normal males (110 $\pm$ 9.5 mg).

The mean weights of the thyroid and adrenal glands obtained from the PTU treated guinea pigs sampled and killed on days 50 and 60 of gestation were greater than those obtained from normal animals of a similar gestational age (Table R8-3).

Histology in PTU treated animals

30 day fetuses - PTU treated

There are no differences in the histological appearance of thyroid glands of 30 day old fetuses from mothers treated with PTU (figures R8-3 and R8-4) compared with fetuses from normal mothers. There are a few colourless 1 μ follicles appearing in the section of the neck region, red cells and connective tissue are also seen.

35 Day fetuses - PTU treated

The follicles are lined by a columnar epithelium and the colloid is stained very faintly (Fig. R8-5) The follicles have an irregular shape the average diameter being 45 ± 2.92 (10-80 μ). This is significantly greater than the follicular diameter in fetuses from normal mothers at this age (16.8 ± 1.5; $P<0.001$).

50 day PTU Thyroid

Macroscopically the goitrous fetal thyroid glands were congested, deep red in colour and softer than usual. The outer surface of the glands had lost its regular shape. Histologically the epithelial cells were hypertrophied. The glands had lost their normal shape and had become convoluted as a result of cellular crowding. No colloid could be seen in the thyroid sections (Figures R8-6 and R8-7).

60 day fetus and day 1-PTU treated

The general form of the gland is totally disrupted. There is a high columnar epithelium and an absence of follicular colloid. Irregular branching tubes of pappillary infolding are seen and there is extreme hypertrophy and hyperplasia of the gland.
(Fig R8-8 and Fig R8-9).

PTU male thyroid

After a long period of PTU treament (50 days) the thyroid glands had a congested colour and were easily distinguished macroscopically. Microscopically hypertrophy and hyperplasia of epithelial cells could clearly be seen. The follicles were surrounded by columnar epithelium and colloid was stained faintly compared to that of normal adult males.

PTU adrenals

The adrenal glands of PTU treated neonates have a thin connective tissue capsule compared to those of normal animals. The zona glomerulosa cells within the clusters are about 10 cells per cluster with large dark nuclei. The middle zone, the zona fasciculata, usually contains vacuolated cells and is about 20-30 cells thick while zona reticularis is about 10-20 cells thick.

Anterior Pituitary of Fetuses Treated with PTU

1. 50 Days

Together with growth in the size of the gland characteristic changes occur in the cellular structure. These changes consist of a sharp decrease in atypical acidophils and basophils at 50 days of experiment in the parenchyma of the anterior lobe and the appearance of vacuoloated cells containg granules and staining like basophils but more lightly than basophils, "basophil-like cells" (Fig. R8-10).

2. 60 days

At 60 days more acidophils and basophils appear and take on their characteristic appearance. The basophil like cells were clearly distinguishable from the basophil and chief cells. There was a sharp derease in the number of acidophil cells compared to those of a normal 60 day fetus (Fig. R8-11).

3. Newborn

The anterior pituitary of the newborn guinea pigs from mothers fed PTU throughout pregnancy are enlarged and there is quite a large number of basophil cells which have a much more widespread distribution in the anterior pituitary. The basophils are usually big with granular cytoplasm and large nuclei. Acidophil cells are also visible and are mainly concentrated in the lateral part of the anterior lobe. The number of acidophil cells is less compared to those found in the anterior pituitary of normal newborn guinea pigs

Skin

Typical hair follicles are seen in the skin sections of 30 day normal fetuses (Fig. R8-12). After birth it was found that hair covered the whole body in PTU treated and normal neonates. Histological sections through the skin showed no differences in the hair follicle structure between the two groups.

Bone

Figures (R8-15) show the cross section through the shaft of long bones obtained from PTU treated and normal neonatal guinea pigs.

A. Normal Bone Sections

It is to be noted that the periosteal surface consists of a cancellous network. This network has formed from a series of longitudinally disposed ridges and grooves. Osteoblast and osteogenic cells cover both sides and the free end of the cancellous bone. The cancellous ossification in the peripheral section appears thicker with narrow blood vessel while in the centre the cancellous ossifications are thinner with large blood filled spaces (Figures R8-13 and R8-14).

B. PTU Bone

The cancellous networks appear only in the periphery of sections with thin ossification and more osteoblast and osteogenic

cells compared to normal bone. In the centre of bone the cancellous network has broken down to individual thin trabeculae (ridges). The trabeculae and cancellous bone are bathed in tissue fluid which is derived from the capillaries of the spaces between the trabeculae. Blood cells appearing in the sections are smaller in size and more in number compared to the blood cells in normal bone (Fig. R8-15).

Radiological Observations

PTU treatment during intra-uterine life led to retardation in the maturation and growth of the newborn skeleton. Macroscopically there was an apparent delay in the appearance of the metatarsals, metacarpals and phalanges of the fore and hind limbs in the goitrous newborn (Fig R8-2). The skull bone was thin and easy to cut compared to normal neonates. Underdevelopment was clearly seen in the histological sections of the long bones and on radiological examination.

1. Appendicular skeleton

There was a lack of ossification of the appendicular skeleton. The length of the long bones e.g tibia, humerus and femur were shorter in the PTU treated neonates. A significant ($P < 0.05$) reduction was only observed in the tibia. Generally the underdevelopment of the epiphyses was clearly seen e.g the distal epiphysis of the femur and the proximal and distal epihysis of the tibia. The epiphyses of these bones were seen to be completely separated from the bone. Delay in the ossification of the olecranon process of the ulna bone was noted.

The underdevelopment may be seen in the radiographs of the short long bones e.g. tarsals, metatarsals, carpals, metacarpals and phalanges. These bones appear as irregular fragmentary areas of opacity whereas in the normal neonates the bones appear as areas of uniform density.

2. Skull and Vertebrae

The skulls of normal neonates were seen to be of more uniform density than those of PTU treated animals where the greater density apppeared only in the maxillary area. In the goitrous young the vertebral column was clearly seen as separated vertebrae while in the normal young the vertebral column appeared as a solid cord of uniform density particularly in the thoracic region. The uniform appearance could be due to the presence of circular masses of fibro-cartillage, the intervertebral discs, between the vertebrae.

Hormone Concentrations

1. Plasma Thyroxine and Triiodothyronine

Table R8-4 summarises the plasma T4 and T3 concentrations in the guinea pigs fed PTU and in untreated animals.

A. 30-35 days In pregnant, PTU treated, guinea pigs the plasma T4 and T3 concentrations at 30 and 35 days were lower than those of corresponding values in untreated guinea pigs.

B. 50 days Maternal T4 and T3 plasma concentrations in PTU treated animals were found to be less (T4 was 11 ng/ml; T3 was 1.06 ng/ml) than those of untreated mothers at the same stage of pregnancy (T4 was 19.4 $\pm$ 0.8 ng/ml and T3 was 2.7 $\pm$ 0.3 ng/ml). In the PTU treated fetal plasma T4 concentration was 11.1 $\pm$ 0.8 ng/ml significantly ($P < 0.0001$) less than the value of untreated fetuses (36.7 $\pm$ 1 ng/ml). T3 concentration in the plasma of PTU treated fetuses was 1.00 $\pm$ 0.03 ng/ml significantly ($P < 0.01$) less than the T3 value (1.53 $\pm$ 0.1 ng/ml of untreated 50 day fetuses (Table R8-4).

C. 60 days The maternal plasma concentration of T4 and T3 in the PTU treated pregnant guinea pigs were 16.2 and 1.8 ng/ml respectively which were less than the T4 and T3 concentrations found in the plasma of untreated guinea pigs of the same gestational age. The T3 concentration in the plasma from PTU treated fetuses was 2.84 $\pm$ 0.2 ng/ml, significantly higher ($P < 0.01$) than the T3 concentration in the untreated fetuses (1.7 $\pm$ 0.1 ng/ml). The T4 concentration was significantly ($P < 0.001$) greater in the untreated fetuses (35 $\pm$ 1.7 ng/ml) than in the treated fetuses (19.1 $\pm$ 3.8 ng/ml; Table R8-4).

D. Neonates Greater differences in the T4 and T3 plasma concentrations were found between the PTU treated and normal neonatal (1 hour old) animals. Plasma T4 concentration was 65.1 $\pm$ 3 ng/ml in the normal animals significantly greater ($P < 0.0001$) than in the PTU

treated neonates (20.6 ± 2.3 ng/ml). Plasma T3 concentration in the untreated neonates was 7 ± 0.5 ng/ml three fold the value in the PTU treated animals (2.3 ± 0.3 ng/ml; $P < 0.0001$).

No differences were found in the mean values of T4 and T3 (24.8 ± 2.4 and 1.9 ± 0.3 ng/ml respectively) measured in the plasma of PTU treated mothers sampled after birth compared with the T4 and T3 concentrations measured in normal mothers at this time (23.3 ± 1 ng/ml and 1.75 ± 0.1 ng/ml respectively).

There was no significant difference between the plasma T4 levels obtained from PTU fed adult males and untreated males while the T3 levels observed in the PTU treated adult males (2.34 ± 0.4 ng/ml) was significantly greater ($P < 0.001$) than the mean T3 level measured in the plasma of untreated adult males (0.7 ± 0.1 ng/ml).

2. Plasma Cortisol Concentrations

A. 30-35 days After combining the data obtained at 30 and 35 days the mean value of plasma cortisol in the mothers treated with PTU was 2350 ng/ml which is almost identical to that measured at 40 days in normal mothers under anaesthesia (Table R8-4).

B. 50-60 days Higher plasma cortisol levels were measured in the PTU fed mothers and their fetuses at 50 days of gestation (Table R8-4). At 60 days of gestation the mean value of plasma cortisol found in treated fetuses was significantly higher ($P < 0.01$) than in normal fetuses. Maternal plasma cortisol levels were higher in PTU fed mothers (Table R8-4). Both groups (PTU treated and normals) were sampled after anaesthesia.

C. Neonatal At the first hour of life the plasma cortisol concentration was 1260 ± 255 ng/ml in the untreated animals significantly ($P < 0.05$) higher than the mean plasma cortisol measured in the neonatal PTU treated animals (823 ± 53 ng/ml). In the mothers equally significant differences were found in the mean plasma

cortisol level measured in the plasma of PTU treated mothers after birth (4362 ± 283 ng/ml) and the concentrations measured in normal mothers (5900 ± 326 ng/ml).

In the males the plasma cortisol levels observed in the PTU treated animals was 314 ± 10 ng/ml significantly greater than the value measured in the normal male ($P < 0.001$) .

Days in gestation	T4		T3		Cortisol	
	Normal	PTU	Normal	PTU	Normal	PTU
30 & 35 Mothers	26.6(3)	16.5 (2)	2.3 (3)	1.6 (2)		2350 (2)
50 Days		****		**		
Fetus	36.7 ± 1.0 (12)	11.1 ± 0.8 (9)	1.53 ± 0.1 (13)	1.0 ± 0.03 (7)	150.0	230.5
Mother	19.4 ± 0.8(7)	11.0	2.7 ± 0.3(5)	1.6	3380.0 ± 277.0(3)	4180
60 Days		***		**		**
Fetus	35.0 ± 1.7(21)	19.1 ± 4.0(5)	1.7 ± 0.1(26)	2.8 ± 0.2(5)	278.0 ± 40.0(5)	570.0 ± 0.0(5)
Mother	21.8 ± 1.6(8)	16.2	2.1 ± 0.3(5)	1.8	3970. 0 ± 233.0(3)	4700
		****		****	*	
Neo-natal	65.1 ± 1.0(17)	20.6 ± 2.3(12)	7.0 ± 0.5(16)	2.3 ± 0.3(7)	1260.0 ± 255.0(8)	823.0 ± 53.0(13)
Mother	23.3 ± 1.0(5)	24.8 ± 2.4(5)	1.8 ± 0.1(5)	1.9 ± 0.3(5)	5900.0 ± 329(5)	4362 ± 283.0(5)
Adult				***		***
Male	25.5 ± 1.3(7)	20.9 ± 0.9(3)	0.7 ± 0.1(6)	2.3 ± 0.4(3)	207.0 ± 10.0(6)	314.0 ± 10.0(3)

Table R8-4. Effect of PTU treatment on fetal plasma T4, T3 and cortisol concentrations.

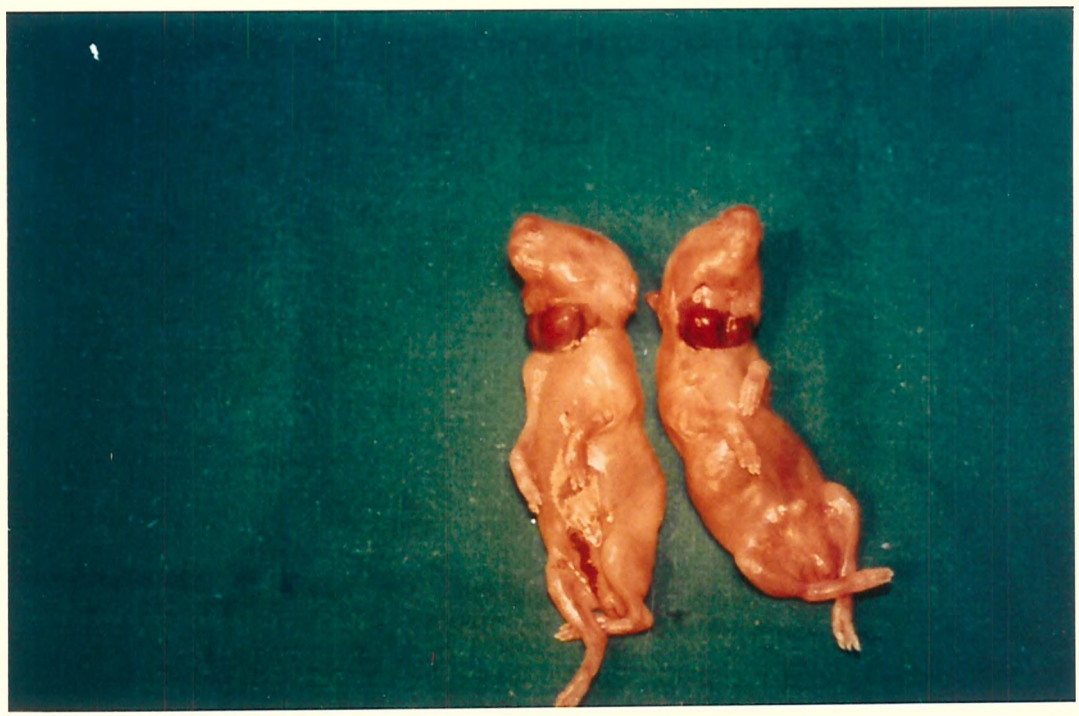


Figure R8-1 Fetuses at 50 days gestational age from PTU treated mothers.

Figure R8-2 Neonatal guinea pigs from PTU treated mothers.

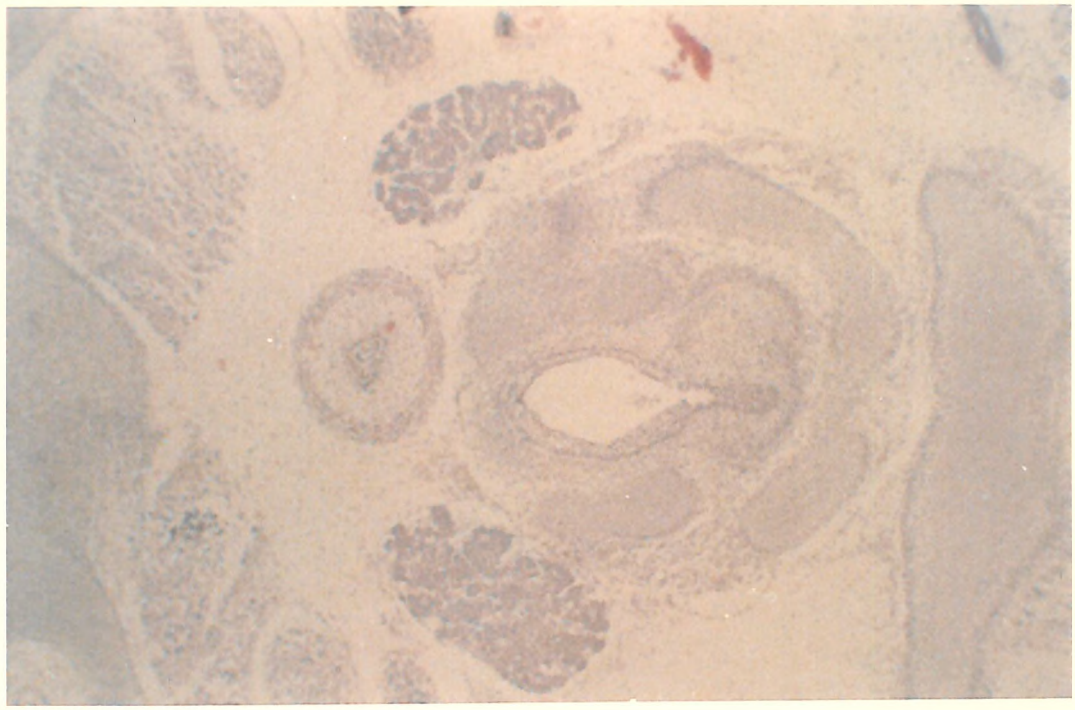


Figure R8-3 Transverse section through part of the neck region to show the thyroid gland in a 30 day old guinea pig fetus from a PTU treated mother. (H&E. X 24).

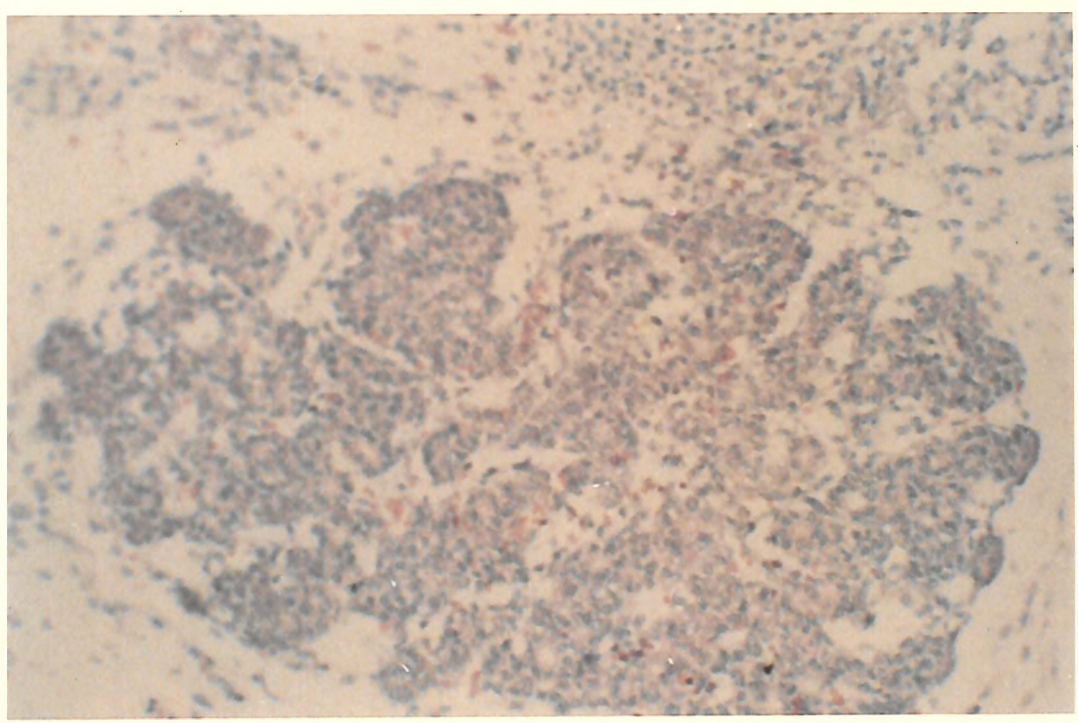


Figure R8-4 Thyroid gland of a 30 day old guinea pig fetus from a PTU treated mother. (H&E. X 96).

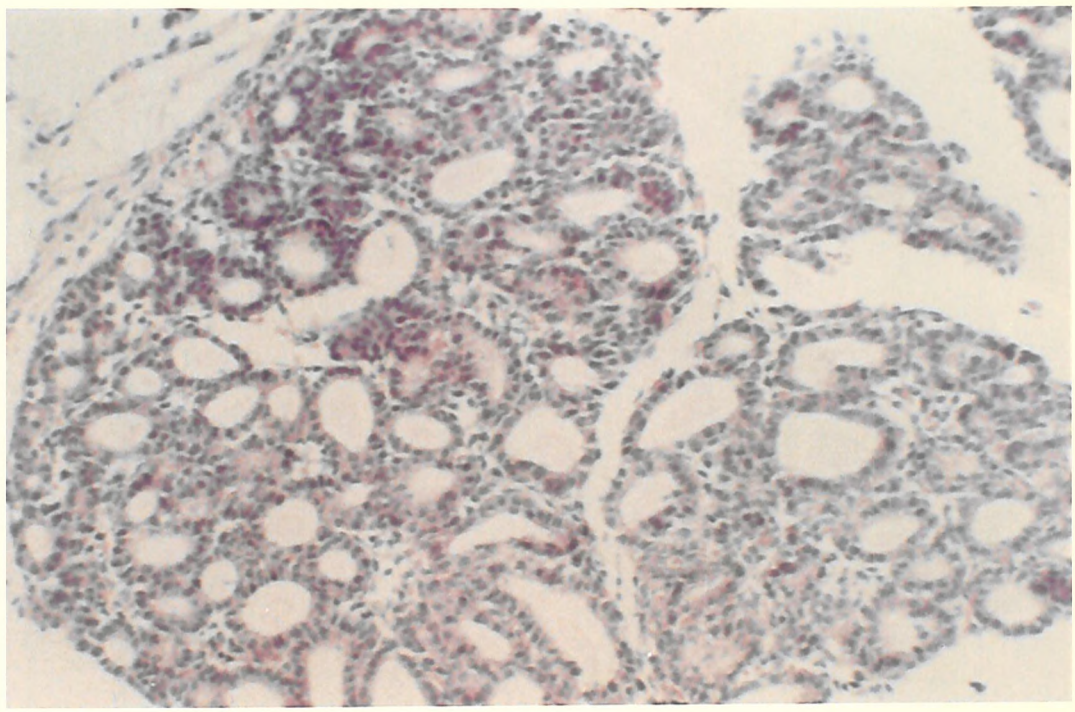


Figure R8-5 Thyroid gland of a 35 day old guinea pig fetus from a PTU treated mother. (H&E. X 101).

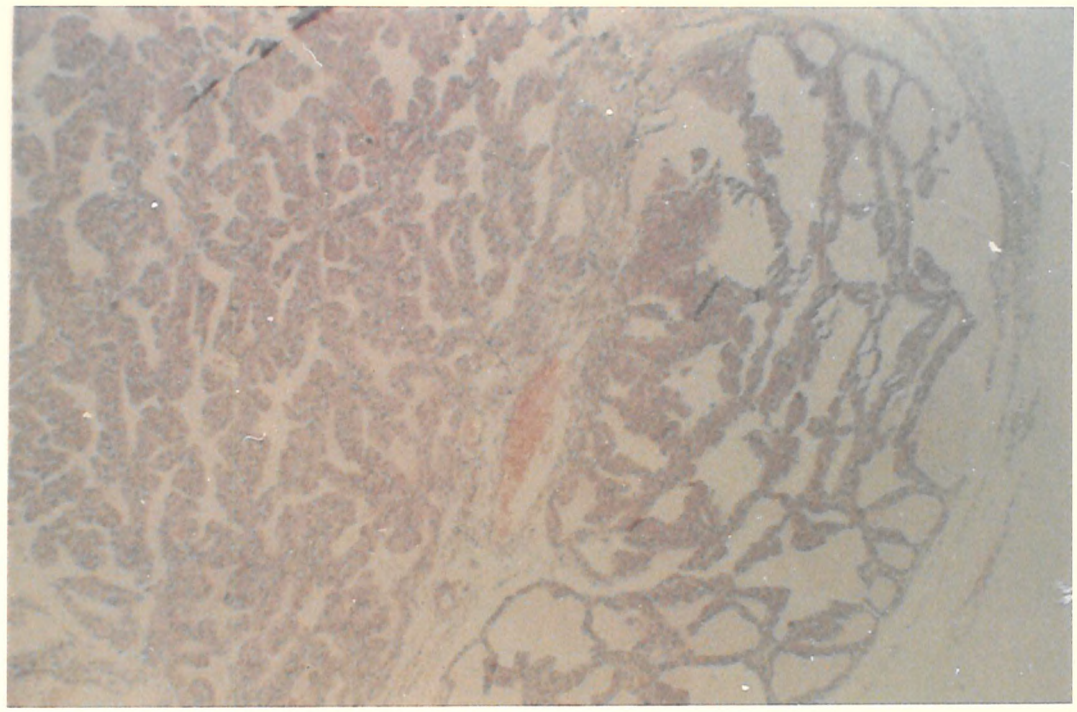


Figure R8-6 Thyroid gland of a 50 day old guinea pig fetus from a PTU treated mother. (H&E. X 23).

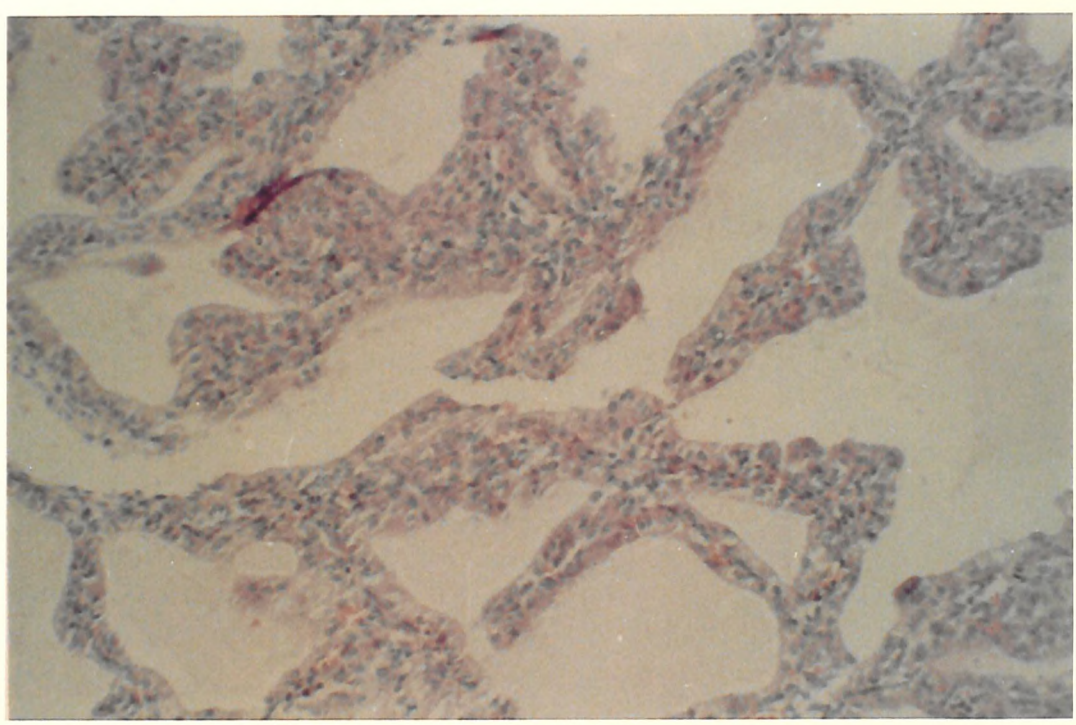


Figure R8-7 Part of the thyroid gland of a 50 day old fetus from a PTU treated mother showing the appearance of areas of irregular pappillary infolding. (H&E. X 96).

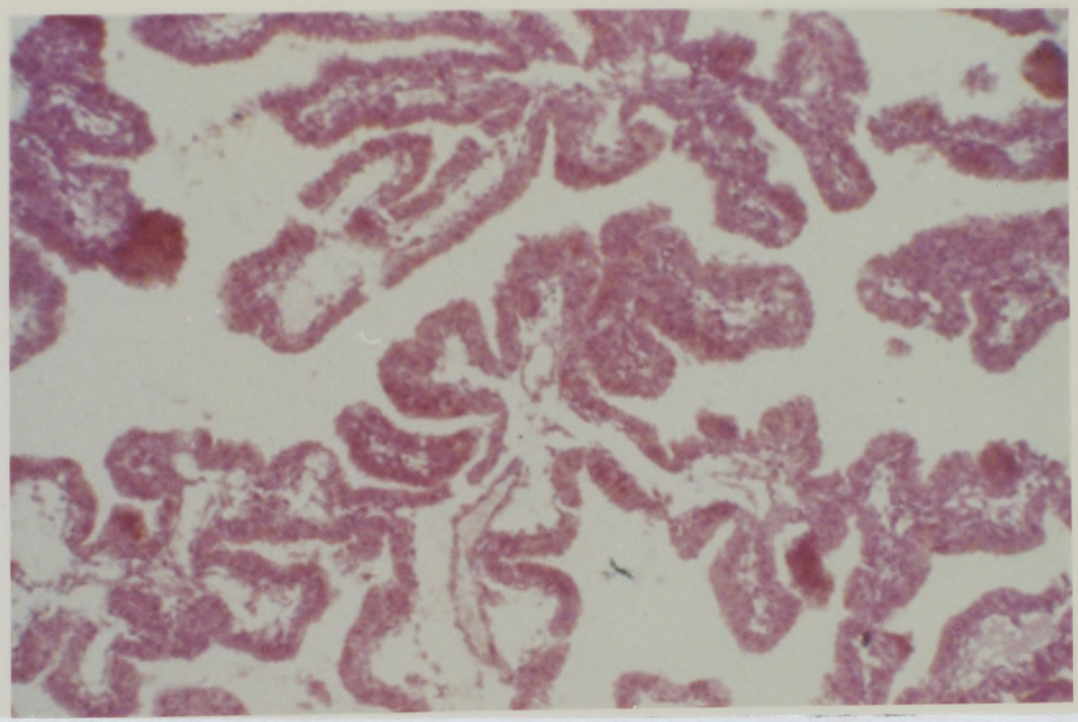


Figure R8-8 Thyroid gland of a 24 hour old neonatal guinea pig from a PTU treated mother. (H&E. x 96).

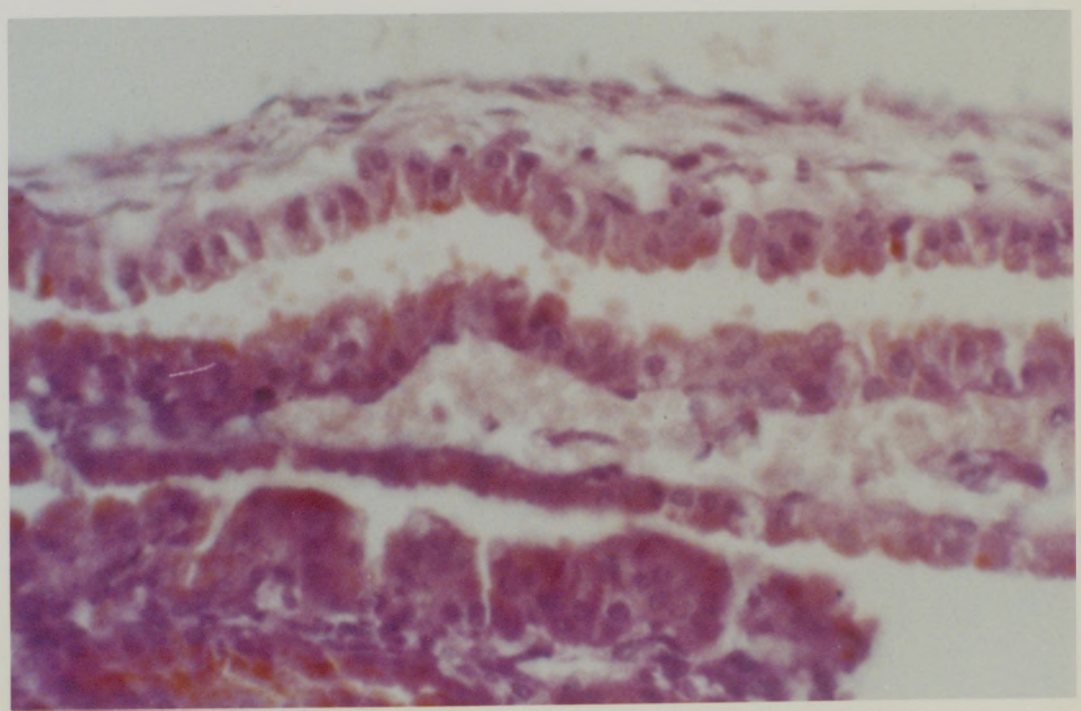


Figure R8-9 Section through the thyroid gland of a neonatal guinea pig from a PTU treated mother showing the high columnar epithelial cells (H&E. X 272).

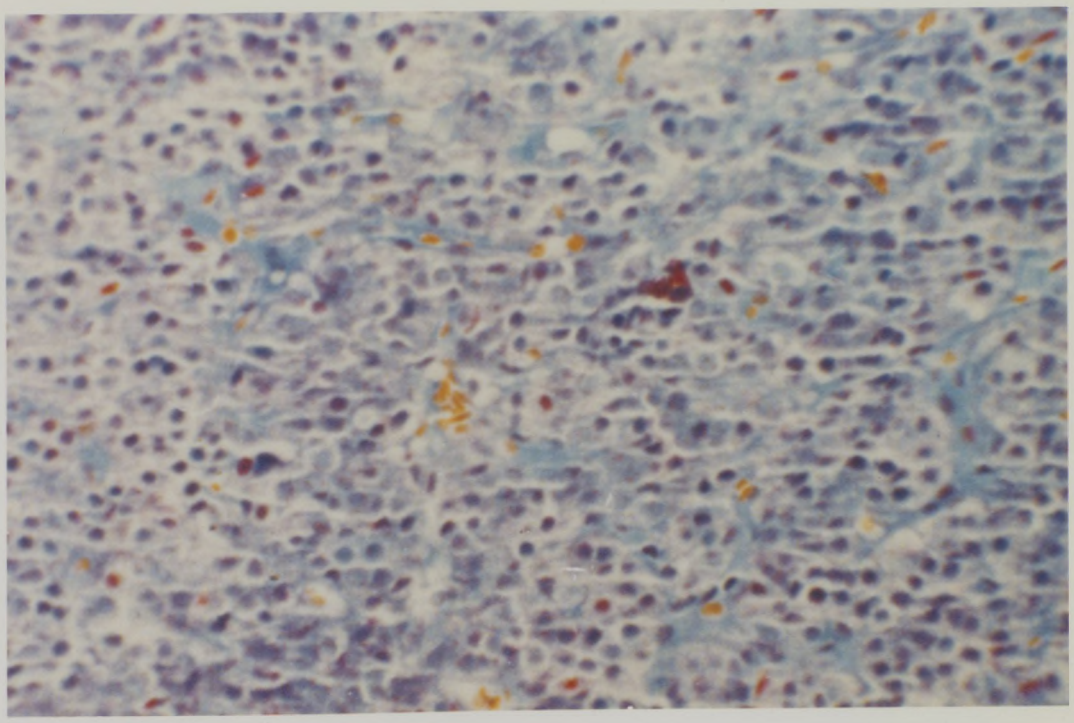


Figure R8-10 Anterior pituitary of a 50 day old guinea pig fetus from a PTU treated mother. (H&E. X 212).

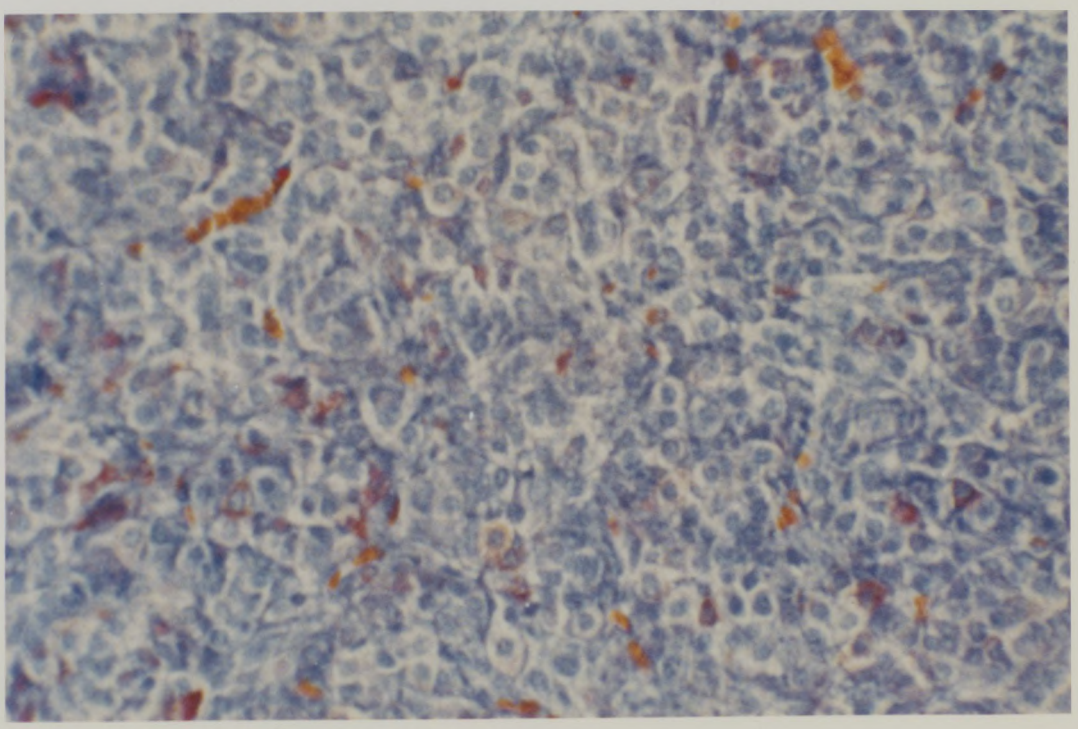


Figure R8-11 Anterior pituitary of a 60 day old guinea pig fetus from a PTU treated mother. (H&E. X 240).

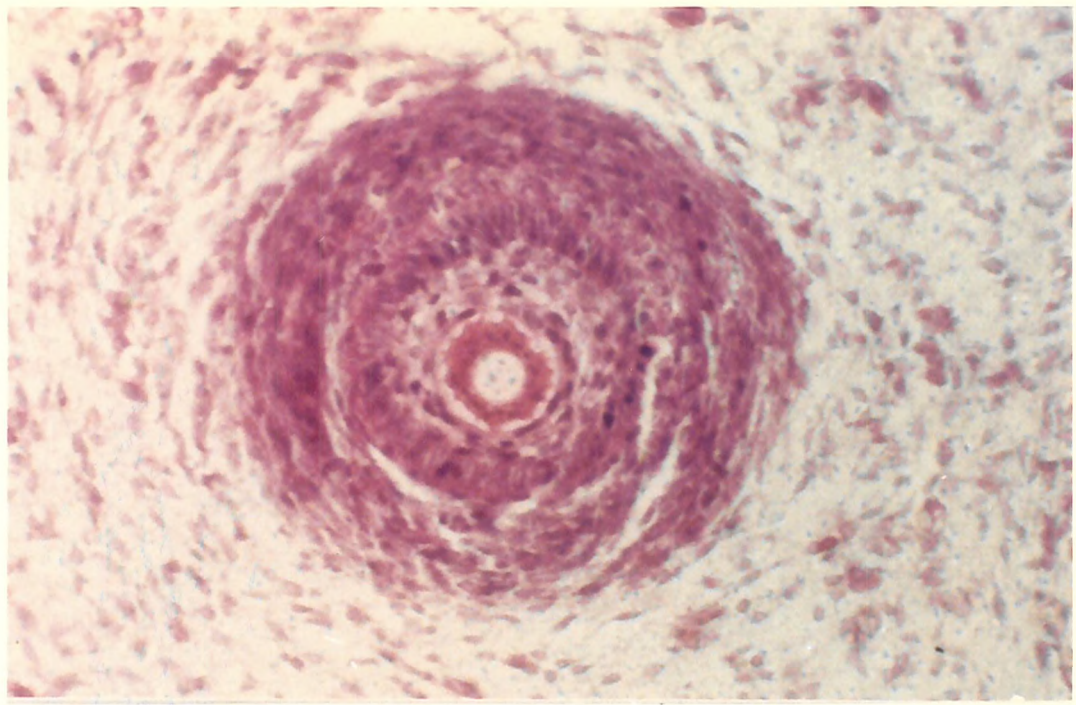


Figure R8-12 Transverse section through the skin of a 30 day old guinea pig fetus showing a typical hair follicle. (H&E. X 240).

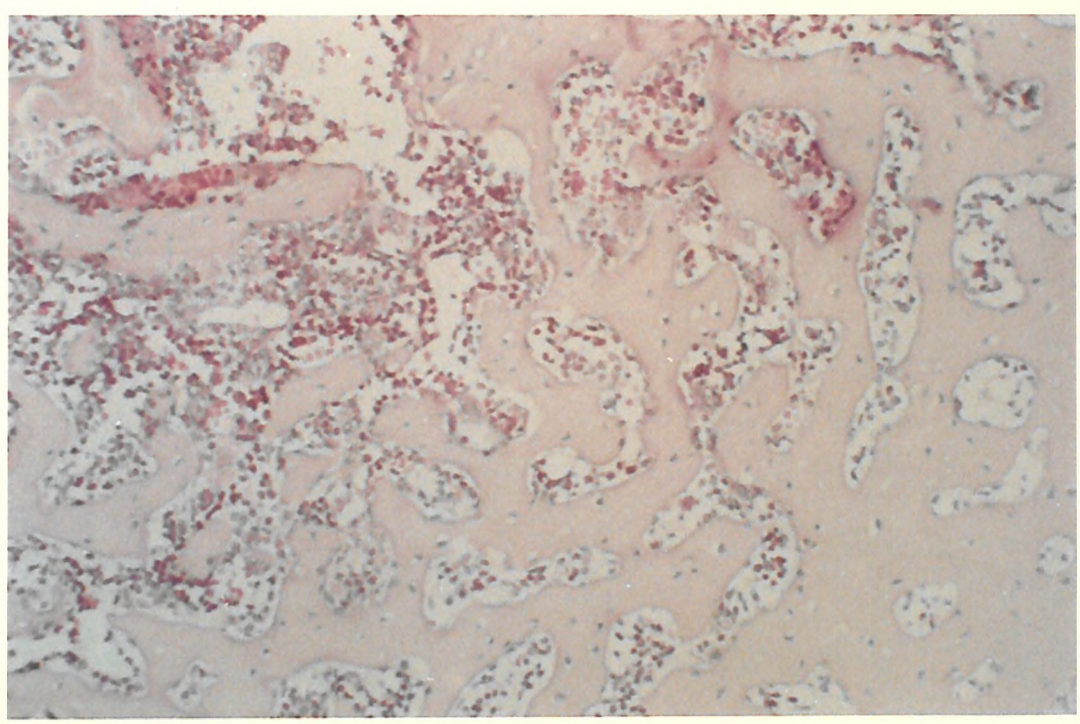


Figure R8-13 Section through a long bone of a 24 hour old neonatal guinea pig from a normal mother. (H&E. X 80).

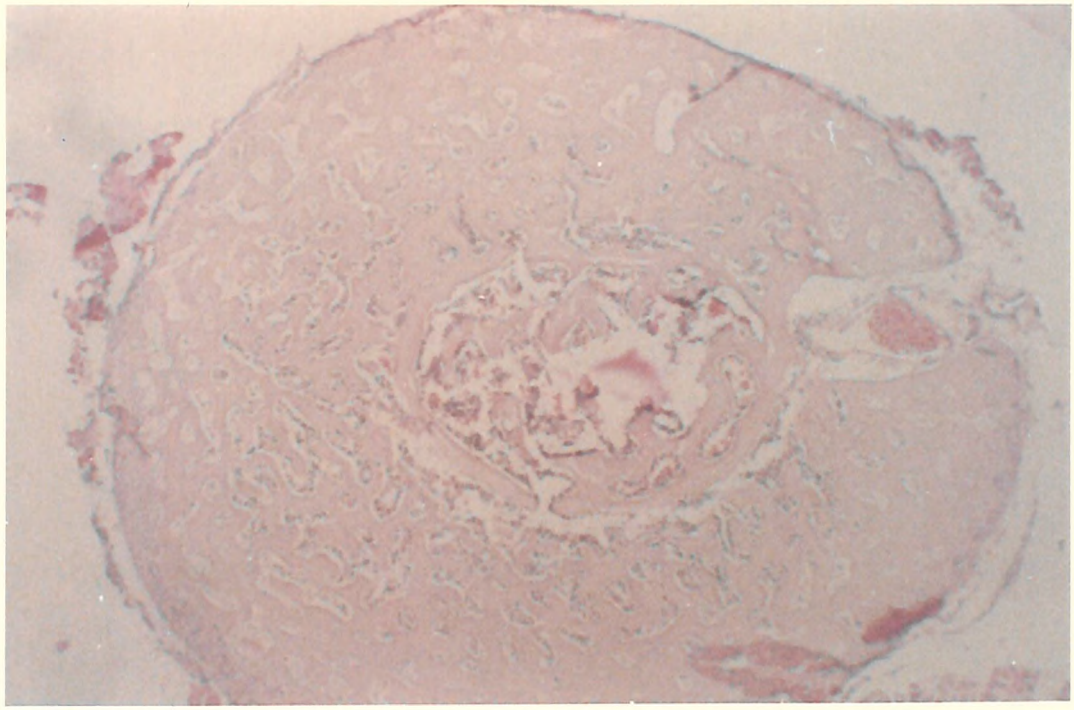


Figure R8-14 Section through a long bone of a 24 hour old neonatal guinea pig from a normal mother. (H&E. X 24.8).

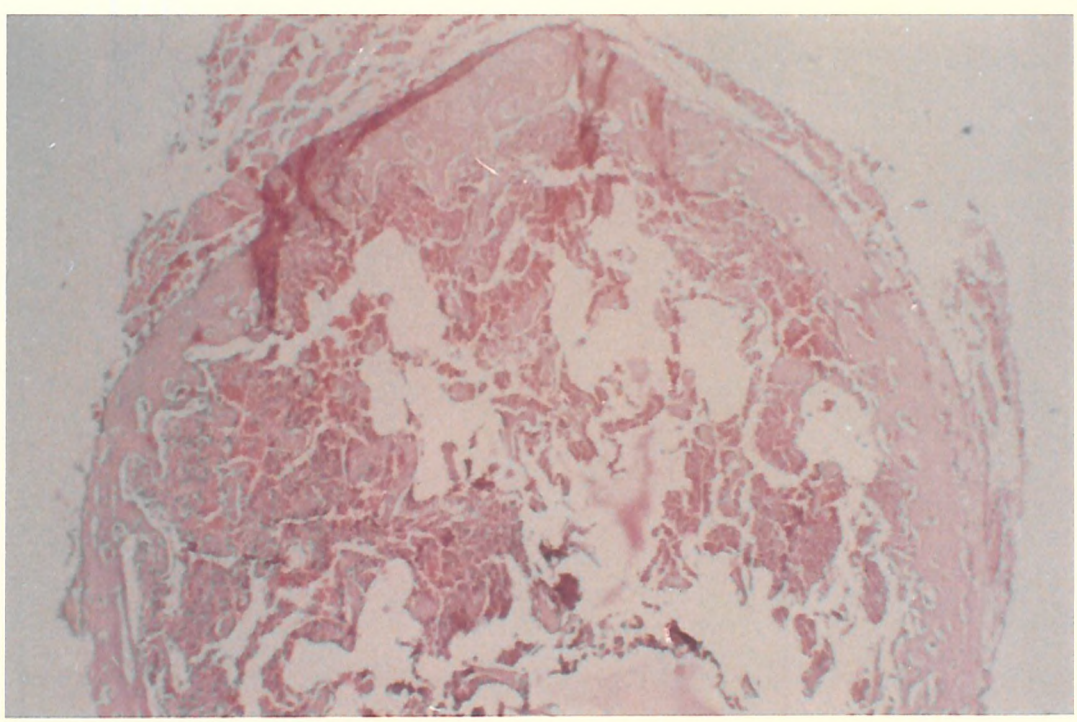


Figure R8-15 Section through a long bone of a neonatal guinea pig from a PTU treated mother. (H&E. X 20.8).

Results Section 9 - Maternal Thyroidectomy

Introduction

Blocking the secretion of fetal thyroid hormones with PTU resulted in massive growth of the fetal thyroid gland and delayed skeletal maturation as well as reduced plasma thyroid hormone concentrations (Section 8). McIntosh, Potter, Mano, Hua, Gragy and Hetzel (1983) showed, in the sheep, that the fetal brain retardation after fetal thyroidectomy alone or in iodine deficiency were less severe than those caused by maternal thyroidectomy followed by fetal thyroidectomy. The effects of lack of maternal thyroid hormones, as a result of surgical thyroidectomy of the mother, on fetal and maternal plasma concentrations of cortisol, T4 and T3 were therefore investigated. The fetal body, thyroid, adrenal and pituitary weights as well as placental weights were also recorded.

Material and Methods

After controlled mating, four female guinea pigs were surgically thyroidectomised at 39 days of gestation under 2% Halothane/ Oxygen/ Nitrous oxide anaesthesia, the tissue removed was fixed for microscopic examination to ensure that it was thyroid tissue.

At 60 days of gestation the animals were killed by a blow to the neck, blood samples were quickly taken by cardiac puncture, fetal carcasses and placentae were removed, blotted on filter paper and weighed. Fetal thyroid, adrenal and pituitary glands were removed, cleaned of surrounding connective tissue and weighed on an Oertling balance. The adrenal and thyroid glands were frozen at -20° c until assay.

Results

Changes in weights

The absolute and relative weights of the thyroid glands in fetuses obtained from thyroidectomised mothers were significantly ($P < 0.01$ and $P < 0.001$ respectively) less than those of normal fetuses. No differences were observed in the absolute or relative weights of the fetal adrenal glands of both groups. The pituitary glands of the fetuses obtained from thyroidectomised mothers were heavier than those from normal mothers but this difference was not significant however the relative pituitary weights were significantly ($P < 0.01$) greater in these fetuses (Table R9-1).

The body weights of fetuses from thyroidectomised mothers were not significantly different from those of intact mothers. There was a significant decrease ($P < 0.01$) in the absolute and relative (g/100g fetal body weight) placental weight in thyroidectomised animals (Table R 9-1 and Fig. R9-1). There were some indications of abnormal development. one fetus was mummified having died before 60 days, another was delivered alive but weighed only 21 gm.

Histology

Fetal thyroid gland

The most striking observation in this experiment was the histological alteration in the thyroid glands of fetuses from thyroidectomised mothers compared with those of normal 60 day fetuses. There was considerable variability in the size and number of the follicles. Many follicles were large and contained no colloid but other, both small and large follicles, contained homogenous purple stained colloid as well as papillary infolding of areas of columnar epithelium as was seen in the thyroid glands of newborn guinea pigs.

Adrenal gland

There were no histological differences between the serial sections of left adrenal glands of fetuses aged 60 days from

thyroidectomised mothers compared with those of normal 60 day fetuses.

Pituitary gland

There were structural alterations in the anterior lobe of the pituitary in fetuses from thyroidectomised mothers. The most numerous cells were the basophils which were scattered throughout the anterior pituitary. Acidophils appeared mainly in the lateral border of the anterior lobe. In fact, the whole picture resembled very closely that of the anterior pituitary on day 1.

Changes in cortisol concentration

Higher plasma cortisol levels were measured in thyroidectomised mothers and their fetuses (Table R9-2). The cortisol concentration in the adrenal gland (ng /100mg adrenal tissue) was significantly ($P < 0.00001$) less in fetuses from thyroidectomised mothers.

Changes in thyroid hormone concentration

Plasma thyroxine concentrations in the thyroidectomised mothers and their fetuses were significantly ($P < 0.001$ and $P < 0.00001$ respectively) less than those of normal mothers and fetuses at the same stage of pregnancy. The maternal plasma T3 concentration in the thyroidectomised mothers was not signifcantly different from that in the normal 60 days old pregnant guinea pigs while the T3 concentration in the plasma of fetuses from throidectomised mothers was significantly ($P < 0.0001$) lower than the mean level of plasma T3 measured in fetuses from intact mothers.

A significant increase ($P < 0.001$) in the T4 concentration (ng/100gm thyroid tissue) was observed in the thyroid glands of fetuses of thyroidectomised mothers compared with normal fetuses of the same age (Table R9-2).

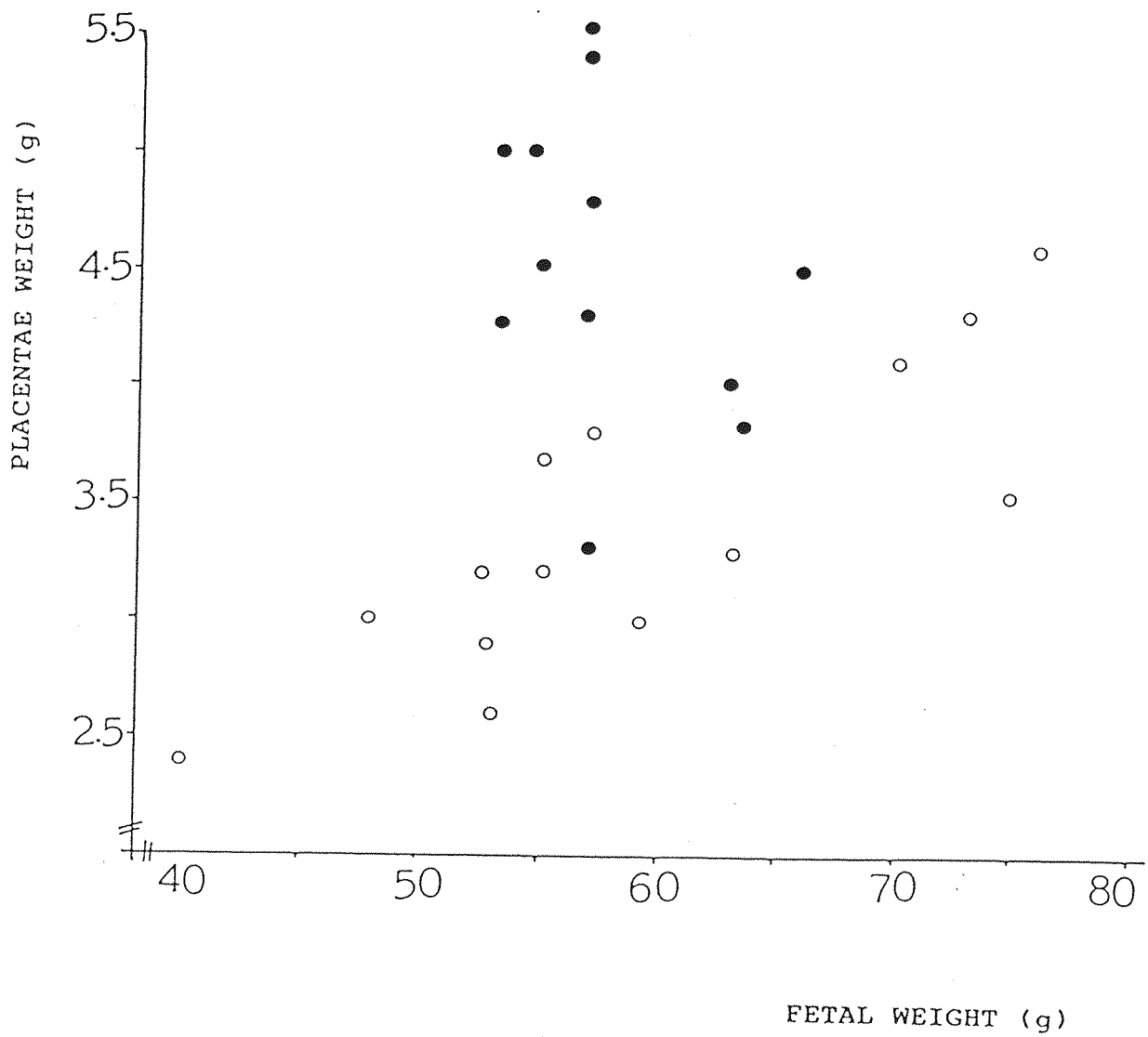
60 days fetuses	Body weight g	Placental mg	Placental g/100g b wt	Thyroid mg	Thyroid mg/100g b wt
Normal (26)	57.00 ± 0.93	4.20 ± 0.12 **	7.36 ± 0.41 **	21.20 ± 0.60 **	37.60 ± 1.16 ***
Treated (14)	55.77 ± 3.72	3.26 ± 0.21	5.92 ± 0.18	16.72 ± 1.32	29.48 ± 1.26

Adrenal mg	Adrenal mg/100g b wt	Pituitary mg	Pituitary mg/100g b wt
9.84 ± 0.47	17.28 ± 0.90	2.42 ± 0.13	4.24 ± 0.25 **
9.31 ± 0.75	16.74 ± 0.67	3.05 ± 0.23	5.58 ± 0.32

Table R9-1. Effect of maternal thyroidectomy on the fetal body weight and the absolute (mg) and relative (mg/100gm body weight) weight of the placenta and fetal thyroid, adrenal and pituitary.

	Fetal		Maternal	
	Normal	Treated	Normal	Treated
Thyroxine	34.90 **** ± 2.00 (21)	15.50 ± 0.52 (9)	21.80 *** ± 1.60 (8)	4.20 ± 0.44 (4)
Triiodo-thyronine	1.70 **** ± 0.10 (21)	0.89 ± 0.09 (12)	2.10 ± 0.30 (5)	2.47 ± 0.24 (4)
Cortisol	245.00 ± 18.00 (12)	345.00 ± 57.97 (12)	3110.00 ± 153.00 (4)	3410.00 ± 868.00 (4)
T4 ng/ mg thyroid tissue	0.99 **** ± 0.10 (6)	2.08 ± 0.05 (10)		
Cortisol ng/mg adrenal tissue	1470.00 12.00 **** (11)	610.00 48.40 (12)		

Table R9-2. Effect of maternal thyroidectomy on fetal and maternal plasma T4, T3, and cortisol concentrations.



FigureR9-1 Influence of maternal thyroidectomy on placental absolute weight (g) at 60 days of gestation in guinea pigs :- Placentae from normal mothers (●), placentae from thyroidectomised mothers (○).

DISCUSSION

Discussion

Iodide uptake

Between 32 and 33 days of gestation radioiodide crosses the guinea pig placenta and appears in the fetal thyroid but there is a significant ($P < 0.001$) increase in fetal thyroid iodide uptake between 32/33 days and 34/35 days (Fig. R5-1 and Table R5-1). Thus 34/35 days can be considered as the earliest time in gestation when the fetal thyroid of the guinea pig might begin to synthesise hormone. According to the present findings the fetal thyroid begins to concentrate iodide before the first appearance of follicles and colloid. This result is in accord with Mitskevich (1962) who concluded that the onset of the fetal guinea pig thyroid functional activity was at about 35 days of gestation. The present data are in agreement with reports for other species, for example iodide is concentrated in the thyroid before the appearance of follicles or colloid in the mouse (Jacobson and Brent, 1959), in the rabbit (Waterman and Gorbman, 1956) and in the calf (Koneff, Nichols, Wolff and Chaikoff, 1949). The earliest time reported for the concentration of iodide in the human fetal thyroid is 70 days of gestation (Fisher et al, 1977). The results reported by Pickering and Kontaxis, (1961) have demonstrated that the thyroid gland of the fetal monkey is actively trapping and incorporating iodide at 50 days of gestation.

Fetal T4

The extraction efficiency of thyroxine from plasma by ethanol was found to be 77.2 % which agrees well with the recovery of 71 % reported by Thomas et al, (1978). The present data concerning plasma thyroxine concentration have not been corrected for recovery. While these experiments were being carried out Jones et al, (1985) and Castro et al, (1986) described the changes in thyroid hormone levels in the guinea pig mother and developing fetus at different stages in gestation. In the present study the concentration of T4 was much higher than that of T3 in the plasma of the fetal guinea pigs.

Thyroxine concentration is progressively increased after 45 days to reach a peak by 55 days and then gradually declines towards term to reach maternal levels at 64 days (Table R4-7 and Fig. R4-1). The T3 concentrations showed similar changes to those of T4 (Fig R4-2 and Table R4-1). The results of the present study agree closely with those reported by Jones et al,(1985) and, less well, with those of Castro et al, (1986). Sufficient plasma could not be obtained from 35 day old fetuses to determine fetal plasma thyroid hormone concentrations. The present data indicate low levels of thyroid hormones in the 40 and 45 day fetal guinea pigs. Fetal plasma T4 concentration is lower in the fetal guinea pig than in the maternal plasma before 45 days of gestation. A similar result was also reported by Castro et al, (1986) in fetal guinea pigs. Low serum T4 concentrations have also been measured in 11-18 week human fetuses (Fisher et al, 1970) These authors comment that the low T4 values are primarily due to low T4 binding protein concentrations. A similar result has been obtained in sheep by Fisher, Dussault, Erenberg and Lam (1972) and Nathanielsz et al, (1973b) and in the pig fetus (Fentener Van Vlissingen et al, 1983). In monkey the fetal plasma T4 values were relatively lower at younger ages (Pickering, 1968). The maternal thyroid is presumably responsible for any thyroid hormone reaching the fetus prior to fetal thyroidogenesis.

In the present study a steady increase in thyroid weight follicle size and colloid content was seen from 45 days onward (Fig. R2-1). Both the increase in plasma T4 and the enlargement of the follicles are most probably TSH dependent. The sudden rise in hormone level after 45 days precedes the increase in follicular size and thyroid weight. The progressive increase in the fetal plasma T4 concentration between 50 and 60 days (Fig. R4-1) is mainly due to an increased T4 secretion from the fetal thyroid gland. The results presented in Table R4-2 demonstrate that the level of thyroxine in the thyroid gland is significantly decreased at 55 days compared to 50 or 60 days of gestation.

Expansion and folding of the follicular walls rather than increased follicle formation characterised the fetal goitres at 50 days of

gestation (figures R8-6 and R8-7) in PTU treated animals. Similar results were also reported by Logothetopoulos and Scott (1956) in guinea pig fetuses at 43 days of gestation. These results indicate that the fetal pituitary-thyroid system is functioning at a relatively high level, resulting in a high fetal T4 secretion rate. Mean fetal plasma T4 concentration exceeds that of the mother at 50 to 62 days of gestation (Table R4-1). This result also indicates that the fetal pituitary-thyroid system is functioning at a relatively higher level than the maternal system at this time. In sheep fetal plasma T4 concentration is higher than that of mother throughout the last third of gestation (Nathanielsz et al, 1973b; Chopra et al, 1975).

The increase in fetal guinea pig T4 concentration coincides with the most important phase of growth and maturation. In the fetal guinea pig the seventh week of gestation has been identified by Flexner (1955) as a critial period of brain differentiation. It has also proved to be a time of intestinal differentiation and, during this period, alkaline phosphatase activity rises 7-8 fold to reach adult levels. These changes are thought to be promoted by increased fetal adrenal activity (Moog, 1979). There are numerous examples showing that an alteration in the timing at which an increase in the fetal plasma thyroid hormones occurs influences the timing of maturation events in the fetus such as a lower rate of fatty acid synthesis in liver or low enzymatic activity in the heart (Jones, 1985).

At 64 days of gestation the decreasing level of fetal plasma thyroxine reaches those measured in the mothers at this time (Table R4-1). The present data do not agree with the results reported by Castro et al, (1986). In the present study the pregnant guinea pigs were sampled at several stages of pregnancy i.e. 40, 45, 50, 55, 60, 62, 64 and 65-67 days while Castro et al, (1986) pooled the data from a group of animals with a mean age of 62 days and a range of 57-65 days. In sheep, a fall in fetal plasma T4 before birth has been reported by Mellor, Matheson, Small and Wright (1976) and Mathur et al, (1980). It has been suggested that such a fall could be caused by changes in the activity of the T4 deiodinase systems at this time or

in the relative metabolic clearance rate of the T4 hormone. In the fetal guinea pig there is an increase in fetal plasma cortisol before birth which results from an increase in the response of the fetal adrenal to ACTH (Table R3-1). It is possible that this increase in cortisol might lead to a decrease in fetal plasma T4 and T3. Since the concentration of cortisol in the fetal plasma rises before parturition the decrease in plasma T4 concentration might be expected to be secondary to the increased cortisol concentration as in the sheep (Thomas et al, 1978). The T4 content in the thyroid gland was decreased after glucocorticoid injection (Table R7-2). Another possibile explanation might be that free thyroxine passage through the placenta increases with advancing pregnancy leading to an equilibrium between the values in maternal and fetal plasma as is reported to occur in the human, rat and rabbit (Osorio, 1960). If this is true in the guinea pig it would explain why the maternal and fetal levels measured in the present study are so similar late in gestation.

Fetal % free T4

A high fetal plasma percentage FT4 (Fig R4-4) was also reported by Castro et al, (1986). These authors only measured fetal % free T4 (% FT4) and free T4 (FT4) at 62 days of gestation and found that the fetal FT4 concentration was about four times the maternal level. This high level of FT4 may indicate that fetal T4 secretion is increaseing more rapidly than the plasma binding protein concentrations at this time. The higher free fatty acid concentration in the plasma of the fetal guinea pig from 55 to 60 days of gestation provides further evidence of a fetal hyperthyroid state. Lipid mobilisation, synthesis and degradation are all accelerated in the presence of excess thyroid hormone (Jones, 1976; Loeb, 1978).

Fetal T3

In the present study plasma T3 was found to be lower in the fetus than in the mother throughout gestation (Table R4-1). The low fetal T3 concentrations are in agreement with those reported by Jones et al,

(1985) but higher than those reported by Castro et al, (1986). Low fetal T3 concentrations have been seen in humans and sheep (Fisher and Odell, 1969; Chopra et al, 1975). However there is a tendency for fetal plasma T3 to rise modestly during the last trimester in humans (Fisher, Dussault, Hobel and Lam, 1973) and a surge in fetal plasma T3 occurs 5 to 6 days before birth in sheep (Thomas and Nathanielsz, 1983). In the guinea pig a significant decrease in fetal plasma T3 was measured at 64, 65 and 67 days of gestation compared to that at 55 or 60 days. This result is again in agreement with the results reported by Jones et al, (1985). Castro et al, (1986) contains no data covering this period. These results indicate that the T3 change in fetal guinea pigs is quite different from that of the sheep and human fetus. The low level of T3 could be due to decreased T3 secretion, decreased availability of T4 for deiodination, or a decreased rate of extrathyroidal conversion of T4 to T3. Castro et al, (1986) have measured a high level of rT3 in the plasma of fetal guinea pigs at 62 days. It appears that conversion of T4 to T3 is reduced in the fetus whereas conversion of T4 to rT3 is well preserved.

The placental inner ring deiodination of T4 plays a part in the regulation of fetal iodothyronine metabolism. Cooper, Gibboens, Thomas and Lowy, (1983) have reported that the placental α-deiodination of fetal T4 could contribute significantly to fetal rT3 levels. Castro, Braverman, Alex, Wu and Emerson (1985) have observed that T3 is actively deiodinated in the inner ring to 3,3'-T2 by the intact guinea pig placenta. A portion of 3,3'-T2 is further deiodinated in the inner ring to generate 3'-T1. No outer ring β deiodination of T3 was seen. A similar high T4, high rT3 and low T3 concentration is found in the fetal sheep, calf and human and all can be explained by a decreased capacity for β deiodiantion of T4 in the fetal liver and perhaps, but not essentially, in other tissues (Thomas and Nathanielsz, 1983). The plasma T3 concentration of fetal guinea pigs is much higher than that of any other species throughout pregnancy. This study suggests that the passage of thyroid hormones through the placenta and the activity of placental inner ring deiodination of T4 and T3 increases in

advancing pregnancy. The higher value of fetal plasma T3 may indicate that in the fetal guinea pig as in the young T3 is important for normal growth and development.

Normal adult T4 and T3

The values of maternal plasma thyroxine measured during pregnancy are similar to those in non-pregnant adult females and adult male guinea pigs (Table R4-3). This observation is in good agreement with the results reported by Castro et al, (1986) for the level of T4 in adult pregnant and non-pregnant guinea pigs. The maternal plasma T3 concentrations reported in this study are elevated during pregnancy compared with those measured in the plasma of adult non-pregnant and adult male guinea pigs (Table R4-3). These T3 data do not agree with the results reported by Castro et al, (1986) in the adult pregnant guinea pig but are in agreement with the levels of T3 these authors measured in adult nonpregnant and adult male guinea pigs. In the present study the assay of T3 was performed with a kit obtained from RIA U.K. In the study carried out by Castro et al, (1986) the T3 assay was performed with ^{125}I T3 supplied by New England Nuclear and a T3 antiserum from Cambridge Medical Diagnostics (Cambridge, Mass. U.S.A.). One possible explanation of the differences between the present data and the data of Castro et al, (1986) might be a cross reaction of the RIA U.K. antibody with rT3 but this was excluded by cross reaction determinations.

In pregnant and non-pregnant adult monkeys Azukizawa et al, (1976) have reported there to be no significant differences in the level of plasma T4 concentration. They observed a rise in the level of plasma T3 in pregnant animals but this rise was not significant. The present observations in the guinea pig are in contrast with the elevated serum T4 and T3 levels found in pregnant women. In human pregnancy there is a progressive increase in serum T4 binding protein (TBG) concentration (Fisher et al, 1970) which is probably due to the high levels of

oestrogen in human pregnancy (Osorio and Myant, 1962). This is probably the reason for the non-significant differences in the plasma T4 concentration between pregnant and non-pregnant female guinea pigs. During pregnancy in the guinea pig there is only a small increase in maternal oestrogen concentration and guinea pig plasma does not contain a specific TBG (Etta, 1973). The difference may also be due to the high cortisol concentration in the plasma of maternal guinea pigs which displaces thyroxine and increases the level of free T4. These high free T4 levels may be responsible for the high FFA concentrations during pregnancy in maternal and fetal plasma (Jones, 1976).

Neonatal Plasma T4

As in other animals the release of thyroid hormone stores at birth results in a maximal plasma hormone concentration within the first day postnatally and then a decrease in the early neonatal period. This increase in plasma T4 concentration is accompanied by an increase in the absolute and relative weight of the thyroid gland and by the appearance of large follicles which have variable shape and contain no colloid. These follicles have areas of papillary infolding and columnar epithelium (Fig. R2-7). All these features indicate that the thyroid gland is very active during the first day of postnatal life. From the second postnatal day onwards plasma T4 levels are decreased (Table R4-3 and Fig R4-1). The observations of postnatal T4 concentrations in this study are in good agreement with results reported by Pals et al, (1973). The sharp rise in plasma thyroid hormones in newborn guinea pigs is similar to that reported for human infants and other species soon after birth. The increased thyroid function in human infants is triggered by an acute release of TSH that rises from 9.4 µU/ml in cord blood to a peak at 30 minutes after birth of 93 µU/ml. This is followed by a decline to 14 µU/ml by 24 hours after birth (Fisher and Odell, 1969). The neonatal calf maintains the level of thyroxine on the first day of life and it then falls rapidly during the neonatal period (Nathanielsz and Thomas, 1973). The lamb resembles the calf in that there is a postnatal rise in plasma T4 (Nathanielsz, 1969). In pigs a similar postnatal surge in the release

of thyroid hormone has been demonstrated in neonatal animals (Slebodzinski et al, 1981).

It has been postulated that the sharp and rapid rise in serum T4 protein-bound iodine in human infants soon after birth is in response to the cold environment into which they are delivered as compared to the uterine environment (Fisher, Oddell and Makoski, 1966). The present data indicate that within the first hours there is an acute stimulation for the release of thyroid hormone as reflected by an about four-fold increase in plasma thyroxine. At about this point further release is shut off by well established feedback mechanisms. The half-life of the decline in plasma T4 in the 30 hours after birth in the present data is 27.7 hours which agrees with the value of 1.23 days (29.5 hours) reported by Pals et al, (1973) and is similar to the value determined for the plasma half-life of T4 in the guinea pig in section 6 (23.42 $\pm$ 2.44 hours). This indicates that little hormone is being secreted at this time. The present results are supported by the marked peak in the thyroidal radioiodide uptake of the neonatal guinea pig immediately after birth which was followed by slow rates of release of labelled hormone (D'Angelo, 1967). Florsheim, Faircloth, Corcorran and Rudko (1966) described a similar peak in thyroidal radioiodide uptake of the newborn rat followed by a precipitous decline during the first postnatal week.

In the guinea pig the rate of decline of the plasma T4 to below the birth level was slower than in the lamb or the calf and more like the human infant. Values declined to the adult level some time after 21 days after birth (Fig. R4-1). The plasma T4 value for the adult guinea pig groups are in agreement with the findings reported by Refetoff, Robin and Fang (1970), Pals et al, (1973) and Castro et al, (1986). It is probable that the low T4 levels in adult guinea pigs in comparison with many other species are due to the fact that the guinea pig has a negligible quantity of specific T4 binding globulin (Etta, 1973).

Neonatal Plasma T3

In the present experiments the plasma T3 level increased sharply between birth and 9 hours in the neonatal guinea pigs. The time course of the increase in T3 in the plasma of the neonates is similar to that of the increase in plasma T4. Plasma T3 continues to rise in the early postnatal period and T3 concentrations do not follow T4 concentrations at this time. The T3 concentration continues to rise during the period in which the T4 concentration falls. The T3 levels remain high for the first 4 days and then decline to twice the adult concentration at 21 days of age (Fig R4-2). These results are similar to those reported by Nathanielsz et al, (1973c) and Fisher et al, (1977) in neonatal lambs. In the newborn calf plasma T3 values are high on the first day of life and fall rapidly in the early neonatal period in a similar manner to T4 plasma concentration (Nathanielsz and Thomas, 1973). In human infants a rise in plasma T3 concentrations was observed during the first 24 hours of postantal life in full term babies (Uhrmann, Marks, Maisels, Friedman, Murray, Kulin, Kaplan and Utiger, 1978). The T3/T4 ratio rises to a high level (Fig. R4-4) immediately after birth and it rises to a maximum value at 30 hours postnatally. Similar results were reported in the newborn calf by Nathanielsz and Thomas (1973).

The postnatal changes in T4 and T3 may be triggered by many aspects of the change from the uterine environment. The mechanism of the post delivery T3 rise is not yet established. It has been suggested that the high postnatal TSH levels results in preferential T3 secretion from the neonatal gland which is responsible for the rise immediately after birth (Brzezinska-Slebodinska and Slebodzinski, 1986; Wu, Polk, Klein and Fisher, 1986). There may be an increase in T3 production as a result of the better oxygenation of the tissues following the onset of respiration but ventilation of term fetuses with intact umbilical circulation does not elevate plasma T3 concentration (Thomas and Nathanielsz, 1983). Delayed cord cutting delays the T3 surge without altering the timing of the TSH surge (Fisher et al, 1977). Nathanielsz

et al, (1973c) have suggested that the sudden increase in plasma T3 after birth does not necesssarily reflect an increased conversion of T4 to T3 but could possibly be due to the absence of placental clearance and metabolism of T3 after delivery.

The rise in the plasma concentration of T3 is linked to an activation of 5' iodothyronine deiodinase enzymes which convert T4 to T3 (Uhrmann et al, 1978). The correlation observed at birth between plasma cortisol, T3 and T4 concentration and T3/T4 ratio support the hypothesis that cortisol could induce the conversion of T4 to T3. In the present study there is little increase in the percentage free T4 in the plasma immediately after birth. In other species this increase in free T4 is attributed to a decreased affinity of the plasma binding proteins for T4 as a result of the high concentration of free fatty acid at this time (Thomas and Nathanielsz, 1983) but plasma free fatty acid concentrations are already high in the guinea pig in late fetal life (Jones, 1976).

The decrease in the plasma concentration of T4 and T3 might be explained by a sharp increase in the peripheral utilization of T4 and T3 (Hillier, 1968) as a consequence of the adaptation of the newborn guinea pig to the extrauterine environment particularly to the new temperature.

Fetal Thyroid Histology

In the present study the development of the thyroid gland is divided into three phases; the precolloid, early colloid and follicular growth phases. In the guinea pig fetus these phases occur at about 30-33 days, 35-40 days and beyond 40 days respectively while in man they occur at 47-72 days, 73-80 days and beyond 80 days (Shepard, Andersen and Andersen, 1964a). In the present study the weight of the thyroid gland in relation to body weight is greater during the precolloid and early colloid periods (Fig R1:4) and there is a decrease in relative weight associated with follicle formation. At this stage (35 days) follicles were very small with little colloid and represented only a small proportion of the gland compared to the interfollicular tissue (Fig. R2-3). This observation agrees with that of Pickering and Kontaxis (1961) in the young monkey fetus. After the beginning of colloid formation the gland only maintains its size in relation to body weight and most of the growth is due to colloid accumulation. These findings concerning the increasing size of the follicles are in agreement with those of Shepard et al, (1964a) in the human fetus. After 40 days there is an increased amount of colloid resulting from an increase in both the number and size of the follicles as gestation advances(Fig. R2-1). Similar observations have been reported in the fetal pig by Fentener Van Vlissingen et al, (1983) who also observed an increase in both size and number of follicles as the fetuses grew.

After birth the relative weight of the thyroid increases to average 34.6 mg/100 g body weight which is greater than that immediately before birth in the 65-67 day old fetus or in the 21 day old young animal (Fig. R1-13). In human fetuses after 80 days the thyroid weight averages 0.045 % of the body weight and this average is close to that of both the newborn (0.049) and the adult (0.036) (Shepard et al, 1964a). A decline in the weight of the fetal thyroid gland relative to body weight during the last third of gestation was also reported in fetal sheep (Nathanielsz et al, 1973b).

The present data suggest that the fetal thyroid gland at 35-55 days of gestation is more active than beyond 55 days. The fetal thyroid gland is more active than that of the young guinea pig and the absolute rate of growth of fetal and newborn thyroid was observed generally to parallel the rate of body weight gain.

Fetal Pituitary Histology

The present data show the fetal pituitary relative weight at 38 days to be about 3 fold that at 65-67 days while the absolute weight at birth is about 4 fold that at 38 days (Table R1:1 & Fig R1:4). The results presented in this study are in good agreement with the data available for the sheep fetus (Mitskevich, 1957). Pituitary weight in the newborn guinea pig is similar to that reported by D'Angelo (1967).

In 40 day old fetal pituitaries the basophils cells are clearly seen especially in preparations stained with M&H but it is also possible to see these basophil cells in anterior pituitary sections stained with H&E. The rapid one-step Mallory-Heidenhain stain described by Casion (1950) is a modification of the longer standard Azan procedure. The one-step M & H technique is simpler, more rapid and consistent and does not require heat whilst the results appear to be the same as those obtained using the longer method (Humason, 1972). Thus the anterior part of the pituitary in the fetal guinea pig displays signs of cytological differentiation at the beginning of the second half of intrauterine life. The differentiation between eosinophls and basophils appears somewhat later. These findings agree in general with those of other studies on pituitary development. In human fetuses cell differentiation can be observed within the anterior lobe by 7 to 10 weeks, pituitary basophils first appeared by 9 weeks and eosinophils are visible by 9 to 10 weeks (Rosen and Ezrin, 1966; Fisher et al, 1977). Alexander, Britton, Cameron, Foster and Nixon (1973) have investigated development of the pituitary of fetal sheep and found that functional activity begins at 54 days when thyrotrophs were identified while corticotrophs were first found at 95 days. TSH

can be identified in serum at 50 days (Alexander et al, 1973; Thorburn and Hopkins, 1973). In rabbits Jost (1953) has shown that the anterior pituitary is very rich in periodic acid-Schiff positive material (FSH and TSH) during the 22nd and 23rd day of gestation.

The results of the present study suggest that the pituitary gland, like the thyroid gland, is active as early as 35-40 days. This supports the observation that the hypothalamus could influence anterior pituitary function through the portal vascular system from 35-40 days of fetal life (Donovan and Peddie, 1973). In rats the pituitary gland is well developed by 17 days and by 18 days two types of granules can be distinguished. Between birth and six days of age there is a progressive increase in the number of granular cells (Glydon, 1957).

Body and placental weight

One of the interesting facts brought out by the present study is that before 30 days of gestation the placenta and fetus are almost the same weight but from then on the fetal weight increases much more rapidly (Table R1-1). It was also noted in the present study that up to the 50 day stage the rate of growth of the fetus is not affected by the number in the litter. In the later stages of gestation the placental and fetal weights are greatly influenced by the amount of crowding in the uterus (Table R1-2 &R1-3). Ibsen (1928) and Eckstein, McKeown and Record (1955) have shown that as the number of individuals in litters increases mean placental and mean fetal weight decreases and they have suggested that in the guinea pig fetal and placental weights are positively correlated. The present data strongly support this suggestion. In human fetuses after 30 weeks both fetus and placenta are heavier for singletons than for twins. In sheep the mean weights of the fetus and the placenta in single pregnancies are heavier than those of twin fetuses and placentae (Robinson et al, 1979). In monkeys early in gestation the placenta grows at a rate greatly in excess of that of the fetus. After 125 days of gestation the monkey fetal weight is 2-3 times as much as that of the placenta (Kerr, Kennan, Waisman and Allen, 1969). In rabbits mean fetal weight/placental weight ratio increased more than twenty fold from the 16th to the 28th day of gestation while the placental blood flow increased ten fold over the same period (Bruce and Abdul-Karim, 1973).

The present results suggest that in the guinea pig uterus the weights of the fetuses are mainly influenced by the number of individuals in the same uterine horn although the fetal weight is also influenced by the number in the opposite horn. The crowding may limit the growth of the placenta which in turn limits the growth of the fetus. These effects could result from competition for the blood supply to the uterus. Fetal growth is affected by the position in the uterine horn, by the maternal age and the number of young in the litter. It is probable that the blood pressure in the uterine artery

will be lower if there are several placentae in a horn and this reduction of blood pressure would influence the rate of maternal blood flow to the placentae and hence could account for the retardation in fetal growth as the number of fetuses increases.

According to Mckeown and Record (1953) a small placenta and low placental blood flow will influence the rate of growth of the fetus. The present study also suggests that the fetal demand for nutrients may affect the balance between placental consumption and transport of nutrients and hence influence placental weight and blood flow especially in the latter stages of gestation when the fetus is growing rapidly. It appears that intrinsic maternal factors exert their main effect in pre-natal life and that prenatal growth is largely controlled by the intra-uterine environment and limited by the mothers ability to supply sufficient space and nourishment to each fetus (Eckstein et al, 1955).

It is possible to compare the mean prenatal and postnatal growth rates of individuals in single, twin and larger litters (Results Section 1). After birth the newborn guinea pig loses weight but by the second day in small litters and by day 4-5 in larger litters (Table R1-4) it has usually regained its birth weight and thereafter grows at a rate which is similar to that seen in late fetal life. Nathanielsz (1969a) found that the newborn lamb exhibits little or no postnatal weight loss. In the human the newborn infant loses weight for a few days after birth but by the tenth day it has usually regained its birth weight (MacKeown and Record, 1953). Similar observations were reported in the newborn calf by Kahl et al, (1977). The changes in growth rate for the newborn guinea pig reported in the present study are different from those of the rat. The newborn rat is born in an immature state after a comparaitively short period of gestation and birth take place before the growth curve reaches its steepest part. There is no measurable break in the rat growth curve at the time of birth (Widdowson, 1968).

It may be of interest to consider the rate of postnatal growth in the various species. This rate is not determined by the state of development at birth for the guinea pig newborn, as reported in the present data, doubles its weight between 14 and 21 days post partum. The human baby doubles its weight in 6 months, the calf in 47 days while the pig and sheep double their weight in 14 days and the rabbit in one week (Harris, 1936-37).

Kinetics

The present study is the first to evaluate thyroxine kinetics in adult guinea pigs. The radioactive thyroxine was mixed with plasma and saline before injection to avoid a rapid increase in non-protein bound radioactive T4 in the plasma immediately after injection. The adminstration of thiocyanate inhibited thyroidal iodide uptake and thus prevented the recirculation of the radioactive iodide label.

The major reason for determining the adult guinea pig thyroxine kinetics was to determine the plasma half life of T4 and compare this with the rate of decline of plasma T4 concentrations in the newborn animals. However values for volume of distribution, fractional turnover rate and T4 secretion rate are also determined. The thyroxine kinetic data from this study (Table R6-1) may be compared with data from the adult male rat since the body shape of the adult male rat is similar to that of the adult male guinea pig although there is some difference in the body weight. The mean body weight of the adult male guinea pigs used in the present study is 1007.5 ± 66.6 g while the mean body weight of rats used by Gregerman and Crowder (1963) was 453 g. Calculation from the kinetic study showed that the mean half-life of T4 in the guinea pig was 23.42 ± 2.44 h while the plasma thyroxine half life in the adult male rat has been estimated by several groups and found to range from 16 to 24 hours. The volume of distribution of T4 in the adult male guinea pig is 197.23 ± 26.39 ml/kg and is similar to the value of 187 ± 10 ml/kg reported by Gregerman and Crowder (1963) in the rat and 122 ± 6.3 ml/kg reported by Silva (1972). The fractional turnover rate (k) was 1.02 per day in the rat (Gregerman and Crowder, 1963) and these authors found the thyroxine secretion rate was 12.2 ± 0.7 µg/kg/day while Silva (1972) reported a value of 8.2 ± 0.2 ug/kg/day. In the guinea pig k=0.73 ± 0.09 per day and the T4 secretion rate was 4.17 ± 0.80 ug/kg/day as estimated here. While the metabolic clearance rate for T4 in the adult guinea pig in present study was found to be 138.25 ± 14.83

ml/kg/day in the adult rat it was found to range from 6.6 to 22 ml/kg/h (Cavalieri, Castle and Mcmahon, 1984; Senga, Pittman, Lindsay, Chambers and Hill, 1982).

In the adult male guinea pig the T4 half life, the volume of distribution and the fractional turnover rate are similar to those in the adult male rat but the T4 turnover is greater in the rat. This difference is mainly due to the higher plasma T4 concentration in the rat. Gregerman and Crowder (1963) report a value of 53.8 ng/ml approximately twice that found in adult guinea pigs (29.2 ng/ml). The basal metabolic rate of the adult rat is about 1.26 ml O_2/g/hr (Silva, 1972) while in the adult guinea pig it is about 0.78 ml O_2/g/hr (Hill, 1958).

Propylthiouracil

Weights

In the present study it is seen from comparison of the data from PTU treated animals and the control animals that up to 35 days of gestation the body weight and placenta weight of both groups are similar (Table R8-11). The histological structure of the fetal thyroids in both groups are also similar at 30 days. These results are in agreement with the observations of Logothetopoulos and Scott (1956). Similar results were reported for the fetal rabbit by Mitskevish (1957) and for the fetal rat (Eguchi et al, 1980). In PTU treated fetuses which were sampled at 35 days of gestation the PTU treatment had induced a significant increase in the relative weight of the thyroid gland and in the size of the follicular cells compared to normal fetuses at 35 days of gestation (Table R8-1 and Fig. R8-5). These results indicate that by day 35 the fetal thyroid was stimulated by elevated pituitary TSH secretion, through a negative feedback mechanism resulting from the fetal thyroid hormone deficiency induced by PTU. Thus the present results support the earlier evidence of the iodide uptake studies that in fetal guinea pig the initiation of hormone synthesis and the relationship between the pituitary and the thyroid is established at the beginning of the second half of gestation, at a time when cords of cells in the normal gland begin to form colloid filled follicles. The organic binding of ^{125}I in the fetal thyroid was found to begin at the 31-32 days stage and the concentration mechanism was well developed by 34-35 days These results constitute a new line of evidence that in the fetal guinea pig the reciprocal relationship between pituitary and thyroid is established at approxiamtely day 34-35 of gestation (see result section 5). In the fetal rat pituitary-thyroid function is established on day 19 or 20 of gestation (Eguchi et al, 1980).

Histology

At 50 days of gestation there is marked fetal thyroid hyperplasia and pituitary hypertrophy (Fig. R8-6 and R8-7), induced by PTU treatment. The present data also show that the fetal goitre is not accompanied by maternal thyroid hyperplasia and this supports the conclusion that the developing fetal thyroid gland is very active and very sensitive to circulating TSH. PTU induces an elevation of the plasma TSH level and an increase of the thyroid weight within 48 hours in adult rats (Griessen and Lemarchand-Berand, 1973). However goitre does not occur in hypophysectomised fetuses but only in intact fetuses of pregnant rats given PTU (Eguchi, Suzuki, Morikawa and Hashimoto, 1971), thus the TSH must be of fetal origin. A difference in placental and body weight between PTU treated and normal fetuses was also reported by Logothetopoulos and Scott (1956) in fetal guinea pigs sampled on day 43 of gestation.

At 60 days the hyperplastic picture first seen at 50 days has developed further and no follicles or colloid can be found in the fetal thyroid glands. The pituitary hypertrophy has also progressed (Fig R8-11). The greater fetal body weight of this group compared with control fetuses of this age probably results from the smaller litter sizes in the treated animals at 60 days(Table R8-1). The greater degree of thyroid enlargemant obtained in this study may result from the duration or route of administration of the goitrogen. Logethetopoulos and Scott (1956) and Ortiz (1959) administered PTU in the drinking water and commenced treatment before mating the animals.

Neonatal Thyroid Weights and Histology

The thyroids of the newborn PTU treated guinea pigs are greatly enlarged (Figures R8-8 and R8-9) and the size of the goitres was closely correlated with the degree of pituitary enlargement and the progressive appearance of M&H positive hypertrophied basophils in the anterior pituitary (Table R8-1). Similar changes in thyroid gland and pituitary weight in the newborn guinea pig were reported by Webster

(1949), Peterson and Young (1952) and D'Angelo (1966b) but these authors did not examine pituitary histology. The present results support the conclusion that the fetal pituitary-thyroid system functions at a higher level than in postnatal life. D'Angelo (1967) has reported that the treatment of newborn guinea pigs with PTU during the first two weeks of life does not produce significant morphological changes in the thyroid or effect TSH secretion. Lascelles and Setchell (1959) have reported similar neonatal goitres after feeding methylthiouracil to pregnant ewes. Eguchi et al, (1980) also reported similar neonatal goitres in the offspring of rats treated with PTU on day 18 or later.

Adrenal Weight and Histology

In the present study there were differences in the absolute and relative weights of the left adrenal gland obtained from the PTU treated fetuses and normal fetuses (Table R8-1) at 50 and 60 days of gestation. These results are associated with the changes in the histology of the pituitary gland after PTU treatment when there is an increase in TSH secreting (basophil) cells and a decrease in ACTH secreting cells. It is likely that the low level of fetal plasma thyroid hormone would result in a slower rate of fetal organ growth and maturation. The newborn PTU treated guinea pigs have smaller adrenal glands (Table R8-1) compared to those of the normal newborn. It is probable that this is at least partly due to the elevated plasma cortisol concentration seen in the PTU treated mothers in late gestation which would suppress fetal adrenal activity. A similar result was reported in fetal rats where the effect was attributed to decreased secretion of ACTH (Lazo-Wasem, 1960; D'Angelo, Stevens, Paschkis and Cantarow, 1953).

Body Weight

In the present data there is no difference in the mean body weight of the treated and normal guinea pigs at birth (Table R8-1). This result is in agreement with that reported by Peterson and Young (1952) and disagrees with the results of D'Angelo (1966b). The differences

between mean weight of the control newborn guinea pigs and the PTU treated guinea pigs which died immediately after birth does agree with the results reported by D'Angelo (1966b). Lascelles and Setchell (1959) reported that the MTU treated lambs were smaller than normal as were the PTU treated neonatal rats of Eguchi et al, (1980). In the PTU treated guinea pigs gestation was prolonged. A similar result was reported by Webster and Young (1948) in the guinea pig and by Lascelles and Setchell (1959) in their study in sheep.

Maternal and Fetal Plasma T4 and T3

No previous information is available regarding plasma thyroid hormone concentrations in maternal or fetal guinea pigs treated with PTU during pregnancy. There is no doubt from the present study and the earlier studies (Peterson and Young, 1952; Logothetopoulos and Scott, 1956; D'Angelo, 1966b and 1967) that placental passage of goitrogens to the fetus occurs and causes inhibition of thyroid hormone formation and stimulation of fetal TSH secretion by the pituitary. There are significant decreases in the fetal and maternal plasma T4 and T3 concentrations (Table R8-4) in the PTU treated animals compared to those measured in normal animals during pregnancy and immediately after birth.

In the present study the levels of plasma thyroxine in the maternal and fetal PTU treated animals are similar to each other at 60 days of gestation (Table R8-4). In normal pregnant guinea pigs and their fetuses the levels of plasma thyroxine at 60 days of gestation on both sides of the placenta are similar (Table R4-1). This similarity between maternal and fetal concentrations in late gestation also seems to be the case in humans, rabbits and rats but not in sheep. In rats there is transplacental passage of T4 from the mother to the fetus (Hamburgh, Sobel, Koblin and Rinestone, 1962; Gray and Gatton, 1974). In the rabbit maternal injection of radioactive T3 and T4 results in high radioactive T4 and T3 concentrations in the fetal serum only in late pregnancy (Hall and Myant, 1956; Myant, 1958). In humans in the last few days of pregnancy the passage of hormone from mother to fetus

is increased (Grumbach and Werners, 1956). In pregnant sheep Hopkins and Thorburn (1971) suggested thyroid hormones do not cross the placenta and the very limited placental permeability to thyroxine in this species which has a syndesmochorial placenta was confirmed by Fisher, Dussault, Erenberg and Lam (1972).

The high level of thyroid hormones in the treated fetuses at 60 days of gestation compared to those at 50 days supports the suggestion that the placenta might be more permeable to T4 at this time. An increase in the passage of hormone across the placenta could be due to an increase in the permeability of the tissues separating the mother from the fetus, to an increase in the surface area of the placental blood vessels or, if blood flow limits the rate of passage of hormone across the placenta, to an increase in blood flow through the placenta.

At 50 days the low level of fetal plasma T4 and T3 is due to destruction of the fetal gland and the limited transfer of thyroid hormone through the placenta. The high level of plasma T3 in treated fetuses sampled at 60 days (Table R8-4) compared to that of the normal fetuses sampled at 60 days might result from a delay in development or may be a result of the higher plasma cortisol seen in the PTU treated animals at this age (see later). Both T3 and T4 are important for fetal organ and body weight development thus the low level of plasma T4 and T3 at 50 days compared to that of normal fetuses will result in delayed fetal maturation.

Beside the placental deiodination of plasma T4 to T3 there is an increase in the T3 passage from the mother to the fetus. If the passage of hormone across the placenta is influenced by the presence of plasma protein binding this would explain why T3 reaches the fetus more readily than T4 since the affinity of these proteins for T3 is much less (Osorio, 1960). Hall and Myant (1956) and Myant (1958) have made observations on rabbits similar to those of Osorio (1960) on rats. After injection of radioactive T3 into pregnant rats the concentration of radioactive T3 in the fetal plasma rose to

equilibrium with that of the mother within 2 hours. The fetal:maternal concentration ratios in pregnant rabbits were determined after injections of radioactive T4 and T3 early in gestation or between the 17th and 30th days. High concentrations of radioactive hormone appeared in the fetal plasma in late gestation but very little in early gestation. The fetal:maternal serum concentration ratio was higher for T3 than for T4. This indicates that the permeability of placenta to T3 is much greater in late gestation.

Neonatal Hormone Concentrations After PTU Treatment

In the newborn PTU treated guinea pig the values of the plasma T4 and T3 are significantly lower than that of the normal newborn guinea pig. They are similar to the concentrations measured at 60 days of gestation in treated fetuses. This suggests that the thyroid of the newborn PTU treated animals is unable to secrete any thyroid hormones (Fig. R8-9). The maternal plasma T4 and T3 levels increased to levels similar to those measured in 66 day pregnant guinea pigs (Table R8-4). This also supports the suggestion that maternal T4 and T3 pass through the guinea pig placenta late in gestation and the placenta play a part in deiodination of T4 and T3. The low proportion of fetal plasma T4 which was free at 50 and 60 days in the PTU treated fetuses compared to the normal fetuses could be due to the low plasma thyroxine concentration or to changes in the fetal plasma protein concentration resulting from the PTU treatment.

T4 and T3 in the adults receiving PTU

The low level of plasma thyroxine in the adult guinea pigs receiving PTU indicates that the PTU at the dose used is an effective agent in inhibiting thyroid function. PTU inhibits uptake of iodide by the thyroid and inactivates thyroid peroxidase and hence disrupts the biosynthesis of the thyroid hormones resulting in low thyroxine formation. The plasma T3 value in the PTU treated adult guinea pigs is higher than that reported in the normal adult guinea pigs (Table R8-4). There may be major changes in the distribution and metabolism

of T3 during PTU treatment. Silva and Mathews (1984) have demonstrated that the plasma T4 clearance rate was increased in hypothyroid rats while the plasma T3 clearance rate was reduced due to a reduction in the T3 volume of distribution. They concluded that an alternative enzymatic pathway, 5'-deiodinase two (5'D-II), is the predominant source of extrathyroidally produced T3 in hypothyroid rats. The 5'D-II is not only responsible for local T3 production in brain and pituitary but also for most of the circulating T3. This second enzymatic pathway of extrathyroidal T3 production is not inhibited by PTU in cerebral cortex and pituitary microsomal preparations but the rT3 5'-deiodinase is inhibited by PTU treatment.

Maternal and Fetal Plasma Cortisol Concentrations

The long-term PTU treatment results in high plasma cortisol levels in the pregnant guinea pig at 50 and 60 days of gestation and even immediately after birth when the stresses of delivery and anaesthesia are also present (Table R8-4). The PTU treatment also caused changes in the metabolism of the adult male and female guinea pigs the effects of which can be clearly seen in the carcasses of the treated animals. The intestines were empty and the internal organs were yellow coloured. The eyes were congested. The mother requires thyroid hormone to maintain the proper environment for the fetus (Osrio, 1960) and there is an increased frequency of abortion and resorbtion of the fetuses when the mother is treated with thiouracil (Krohn and White, 1950). All these factors could help explain the high value of plasma cortisol measured in the PTU treated mothers and their fetuses in this study.

The high level of plasma cortisol in the PTU treated fetuses compared to those of normal fetuses results from the high maternal plasma cortisol in the PTU treated animals. The changes in the maternal and fetal plasma cortisol during pregnancy are discussed elsewhere. The high values of plasma cortisol at each stage of gestation, at parturition and in adult guinea pigs are in accord with the results reported by D'Angelo (1966b). In the present study the

administration of goitrogen did not influence the adrenal hypertrophy of pregnancy nor affect adrenal size in non-pregnant animals. Similar results are also reported by D'Angelo (1966b) in guinea pigs.

Neonatal Plasma Cortisol

In the PTU treated newborn sampled immediately after birth the mean value of plasma cortisol is significantly lower than that measured in the normal newborn guinea pig sampled immediately after birth (Table R8-4). D'Angelo (1966b) also measured lower concentrations in PTU treated compared with normal newborn guinea pigs but he measured lower cortisol levels using Silber's fluorometric procedure which is very non-specific. In the present study an RIA with a highly specific cortisol antibody has been used. A moderate atrophy of the adrenal gland was also reported by D'Angelo (1966b) in newborn guinea pigs born to PTU treated mothers. In the present study the moderate atrophy of the adrenal gland and low level of the plasma cortisol in the PTU treated newborn guinea pigs is attributed to decreased secretion of ACTH during fetal life. The differences in the histological appearance of the normal and PTU treated animals support the suggestion of a shift in pituitary production from corticotrophin to thyrotrophin in these animals. Fetal ACTH secretion will be inhibited by the higher than normal quantities of cortisol crossing the placenta from the mother. After birth the suppply of maternal cortisol is missing and the small neonatal gland is unable to make up the deficit.

Effect of PTU on Bone and Hair Development

In these experiments the development of the fetal hair follicles is not retarded by PTU treatment (Fig. R8-12). This result is in contrast with the observation of Hopkins and Thorburn (1972) following thyroidectomy of fetal sheep. It seems that in the guinea pig fetal plasma thyroid hormones are not essential for the initial hair follicle development. Thorburn, Waters, Young, Dolling, Bunting and

Hopkins (1981) found that the integument effects of fetal thyroidectomy could be reversed by giving epidermal growth factor (EGF) to the fetus. The lack of effect of PTU on fetal skin development suggests that EGF may be secreted by cells within the thyroid gland since these would probably not be affected by PTU treatment. However thyroid hormone deficiency does result in underdevelopment of the epiphyses of the long bones and a delay in ossification in long and short bones (FigR8:1). The comparative study of the histological sections from the normal and PTU treated newborn guinea pigs support this conclusion (Figures R8-14 and R8-15). A similar finding was reported during fetal hypothyroidism in sheep by Lascelles and Setchell (1959) and Hopkins and Thorburn (1972). Reduction was seen in the growth and maturation of the monkey fetal skeleton after ^{131}I injection on the 86th day of pregnancy (Kerr et al, 1972). PTU treatment causes a retardion in the degree of ossification in fetal rats (Eguchi et al, 1980). Wesis and Noback (1949) have also shown that if the mother is treated with thiouracil ossification in the rat fetus is delayed.

Cortisol

The present results provide further information about changes in the plasma cortisol concentration in mothers and fetuses from mid-gestation until delivery and in the newborn and adult male and female guinea pigs. It has been shown that anaesthesia and surgery increase the cortisol concentration measured in plasma from guinea pig mothers throughout gestation but only in fetal samples obtained a few (3-5) days before term (Table R3-1 and Fig.R3-1). The differences in the levels of cortisol in non-anaesthetised mothers and in anaesthetised mothers in this study was similar to that observed in pregnant guinea pigs under the same conditions in late gestation, 62 days until term, by Dalle and Delost, (1976) and (1979) and by Dalle et al, (1983). The high levels, compared to non-pregnant animals, of plasma cortisol measured in 30 to 60 day pregnant guinea pigs killed by a blow to the back of the neck and the higher cortisol concentrations measured towards term were also reported by Hoar (1965) and Jones (1974). The rise occurring during pregnancy in the maternal plasma cortisol in guinea pigs is similar to that seen in pregnant Rhesus monkeys (Kittinger, 1974) and pregnant women (Rosenthal, Salunwhite and Saudberg, 1969). There is a similar increase in corticosterone levels in the pregnant rat (Kamoun, 1970). In the ewe the increase in plasma cortisol level begins only 4 to 5 days prior to parturtition (Bassett and Thorburn, 1969).

The maternal plasma cortisol concentration remains high and mainly constant towards the end of pregnancy and the maternal levels are higher than those of their fetuses (Table R3-1). Combined with the increase in maternal adrenal weight during pregnancy this suggests that there is marked adrenal stimulation during gestation. Dalle and Delost (1979) showed that the maternal adrenals are the principal source of maternal plasma cortisol at this time. The experiments of Dalle et al, (1983) support this suggestion. The increase in maternal plasma cortisol concentration, and in the concentration of cortisol in the liver which is equal to the plasma concentration is associated

with a very low maternal MCR when compared with the MCR measured in non-pregnant guinea pigs under the same conditions (Dalle et al, 1983). In addition to the continuous increase in unbound cortisol concentration seen during pregnancy (Gala and Westphal, 1967) there is a very high corticosteroid binding globulin, (CBG) concentration (Diamond, Rust and Westphal, 1969). All these factors contribute to maintaining the high maternal cortisol concentration during pregnancy.

A rise in CBG during pregnancy was also found in women (Willcox, Youich, McClom and Schmitt, 1985), and rabbits (Mulay, Giannopoulos and Solomon, 1973). In Rhesus monkeys (Kittinger, 1974) and sheep (Beitins, Kowarski, Shermata, Delemos and Migeon, 1970) the levels of CBG do not increase significantly during pregnancy and the MCR of cortisol remains high.

Fetal Cortisol

The high levels of fetal plasma cortisol between 50 and 60 days has also been reported by Jones (1974) and by Jones and Roebuck (1980). There are no significant differences in the plasma cortisol concentrations of fetuses at 50 to 60 days of gestation taken from mothers under sodium pentobarbitone anaesthesia or those killed by a blow to the back of the neck (Fig.R3-2). The present result is similar to that observed in fetuses of the same age and under the same conditions by Jones and Roebuck (1980). Jones (1974) reported higher levels of fetal plasma corticosteroids but in his study a relatively non-specific competitive protein binding assay (CPB) was used rather than a specific radioimmunoassay. This method (CPB) measures any compound which will bind to the binding agent, e.g. progesterone and corticosterone also bind to CBG and may give an over estimation of the cortisol concentration. After 60 days until delivery similar levels of fetal plasma cortisol to the present results are reported by Jones and Roebuck (1980) in fetuses under the same conditions but the present study shows higher levels of plasma cortisol in fetuses taken from anaesthetised mothers from 64 days to term than those reported by Jones and Roebuck (1980). The concentrations measured at this age are much

lower than the cortisol levels reported by Dalle and Delost (1976 and 1979) and the plasma corticosteroids measured by (Jones 1974) under the same conditions. These differences are probably partly related to differences in the sampling procedure but are probably mainly due to the higher specificty of the assay for cortisol used in the present study.

The fetal adrenal cortisol concentrations are very high at 35 days and decrease significantly by day 50 of gestation (Fig. R3-4). This observation has also been reported by Hoar (1965). The adrenal cortisol concentration increased again to reach a second peak at 60 days before sharply decreasing towards term. These observations agree with those of Dalle and Delost (1976) but not with Hoar (1965) who reported a high level at 35 days which then gradually decreased until day 65. These changes in the adrenal gland cortisol concentration can be explained as follows. The increase in weight of the adrenal gland from 35 days to 50 days is not associated with an increase in cortisol production rate. The fetal adrenal is unresponsive to ACTH stimulation at 46-55 days and responds very little at 56 to 60 days (Jones and Roebuck, 1980 and 1980a). They have reported "ACTH big peptide" suppresses the action of ACTH on adrenal cells between 55 and 60 days. Low ACTH concentrations in fetal guinea pig plasma result from a lack of pituitary maturation before sixty days (Jones et al, 1985).

Fetal cortisol is largely of maternal origin before 60 days (Dalle et al, 1983). The adrenal gland of the fetal guinea pig is capable of responding to ACTH stimulation as early as 60 days in vivo (Donovan and Peddie, 1974; Jones and Roebuck, 1980 and 1980a) and much of the fetal cortisol is of fetal origin after this time (Jones, 1974; Dalle and Delost, 1979; Dalle et al, 1983). This increase in response to ACTH by the fetal adrenal in late gestation is similar to that reported in fetal sheep (Liggins et al, 1973), in fetal pigs (Dvorak, 1972), in fetal calves (Comline et al, 1974), in fetal horses (Nathanielsz et al, 1975) and in fetal rabbits (Jost, 1966). The increase of the adrenal gland cortisol concentration between 55 and 60 days coincides with structural changes in the adrenal cortex which indicate increased

steroidogenic activity (Black, 1972). The fetal plasma cortisol concentration increases after 60 days of gestation partly as a result of the increased fetal adrenal activity described above but the fetal plasma cortisol concentrations remain lower than the corresponding maternal plasma cortisol levels (Fig. R3-1). Similar results have been reported in the guinea pig by Dalle and Delost (1976 and 1979), in rhesus monkeys by Kittinger (1974) and in sheep by Beitins et al, (1970).

The important differences in the rate of cortisol transference across the placenta in the guinea pig, human and monkey (Anderson and Winter, 1985) in comparison with those in the ewe and goat (Nathanielsz, 1976) can be explained partly by the differences in the placentae of these species. The haemomonochorial placenta of the guinea pig has numerous vascular anastomoses between mother and fetus which increase during the last days of pregnancy and this increases the transport of cortisol across the placenta as gestation progresses (Firth and Farr, 1977). A similar observation has also been reported in monkeys (Kittinger, 1974). In sheep no marked change in the placental permeability occurs until about 10 days before delivery (Nathanielsz, 1976). Dalle et al, (1983) have reported high activity of placental 11 β hydroxysteroid dehydrogenase, the enzyme which transforms cortisone into cortisol, in the guinea pig during the last days of pregnancy. This has also been reported in the rabbit (Barr, Lugg and Nicholas, 1980), sheep (Thomas, Wilson, Pierrepoint, Cameron and Griffiths, 1976), monkey (Pape and Aibrecht, 1984) and human (Murphy, 1979). Thus towards the end of gestation the fetus will be more capable of converting cortisone from the mother into cortisol. The increase in the concentrations of CBG and free cortisol in fetal plasma prior to term has been reported in the guinea pig by Jones (1974) in the sheep by Liggins et al, (1973) and in the rabbit by Mulay et al, 1973).

The high level of fetal plasma cortisol in the last days of gestation can also be explained by a low cortisol metabolic clearance rate (MCR). Dalle et al, (1983) reported a low cortisol MCR in the

mother and a much lower cortisol MCR in the fetus due to the low enzymatic activity in fetal liver and also because fetal adrenal tissue is not able to catabolise its own secreted cortisol as the maternal adrenal does (Greiner, Kramer and Colby, 1976).

The increase in the level of fetal plasma cortisol can be summarised as follows :-

1. The rapid increase in the adrenal absolute weight and the structural changes in the adrenal explain the increase in the cortisol production rate.

2 An increase in the placental transport of cortisol combined with an increase in activity of 11 β hydroxysteroid dehydrogenase which increases the transformation of cortisone to cortisol

3 An increase in the fetal CBG concentration combined with a low fetal MCR.

Newborn

In the present study (Fig. R3-3) the high plasma concentration of cortisol, the main glucocorticoid, measured in the newborn guinea pigs corresponds well with the results described by Malinowska and Nathanielsz (1974), Malinowska, Hardy and Nathanielsz (1972a and 1972b) and Dalle and Delost (1974). The extremely high plasma cortisol concentrations immediately after birth may originate from either the maternal or fetal adrenal. Evidence for the first possibility exists in that the placenta is permeable to cortisol in the guinea pig and the maternal levels of cortisol at the time of parturition are very much higher than those of the fetus. The fetal adrenal activity may thus be partly inhibited by maternal steroid secretion during the last days of pregnancy. The second possibility is supported by the observation that adrenal cortisol concentrations increase immediately after birth (Fig R3-3). Thus it appears that the adrenals of the newborn guinea pig are capable of responding to the stress of parturition. After stress plasma cortisol levels are significantly increased in the adult guinea pig (Hani, Dalle and Delost, 1980; Leppaluoto et al, 1981).

At birth it has been found that plasma corticosteroid concentrations are elevated in human infants (Stevens, 1970) the lamb and calf (Bassett and Alexandra, 1971, Nathanielsz et al, 1972) rat and rabbit (Malinowska et al 1972a; Cohen 1976; Fullerton, 1983) and pig (Dvorak, 1972).

In the newborn guinea pig the present results indicate the half-life of the decline in plasma cortisol level (not the half-life of plasma cortisol) during the first 48 hours of life is 69.3 hours which is much longer than the plasma half-life of cortisol in the adult. Such an observation suggests that immediately after birth the neonatal guinea pig is secreting cortisol into the circulation rather than clearing cortisol which had previously crossed the placenta from the mother. A similar finding of a long half life of decline of plasma concentration in the new born immediately after birth was found in the rat (Malinowska et al 1972a) and human infant (Bongiovanni, Ederlein, Westphal and Boggs, 1958).

The fall in plasma cortisol occurs essentially between 24hr and 21 days after birth (Fig. R3-3). After 21 days the concentration of cortisol is similar to the low levels found in the adult. As in the pig (Dvorak, 1972) the guinea pig establishes the adult concentration of its principal glucocorticoid more slowly than in some other species. In man this concentration of cortisol is reached on the second day (Haugen, Brinck, Johusen and Knutrud 1967; Stevens 1970), on the seventh day in sheep (Paisey and Nathanielsz, 1971), and on the first day in the rabbit (Malinowska et al, 1972a). The high levels for plasma cortisol between birth and six days correspond with the high concentrations in the adrenal glands. There was an increase in adrenal corticosteroids in the male from 24 hours to 50 hours and from the sixth to seventh days.

A decline in peripheral plasma cortisol levels in adult male and female guinea pigs has also been noted by Fajer and Vogt (1963), Dalle and Delost (1974) and Greiner et al, (1976). The observations of Fajer and Vogt (1963) suggested that the fall results from diminished adrenal

secretion. Cortisol secretion was greater in females than males and 11β-hydroxylase activity, the final enzymatic step in cortisol production was lower in mature than immature guinea pigs. Also the MCR was significantly higher and CBG concentration was significantly lower in adult guinea pigs (Hani et al, 1980). These factors could explain the lower levels of plasma cortisol measured in the adult guinea pig. Decreasing neonatal plasma corticosteroid concentrations were also reported in the goat (Riegle, Przekop and Neller ,1968) the bull (Riegle and Neller, 1967) and the rat (Cohen, 1976; Fullerton, 1983)

Adrenal Weight

Fetal

In the fetal guinea pig the absolute weight of the left adrenal gland is increased steadily throughout gestation but shows no sharp increase near term (Fig. R1-5). This finding is similar to that reported in the same species by Illingworth et al, (1974) and by Moog and Ortiz (1960). In humans the fetal adrenal glands grow steadily and reach maximum weight (relative to body weight) between the twelfth and seventeenth week (Jost, 1975). In the rat fetus the adrenal stops growing on the day before birth. This finding contrasts with that in fetal lambs (Comline and Silver, 1961; Boshier, Halloway and Liggins, 1980), pigs (Dvorak, 1972) and monkeys (Kerr et al, 1969; Mcnulty, Novy and Walsh, 1981) where the adrenal weight increases greatly during the last 10 to 14 days of gestation.

In this study the relative weight of the fetal guinea pig adrenal gland shows two peaks (Fig. R1-4). The higher peak at 35 days and a second peak at 50 days. After 50 days the relative weight (mg per 100 g body weight) of the left adrenal glands showed a negative correlation with body weight. Illingworth et al, (1974) and Moog and Ortize (1960) also reported a negative correlation between the relative weight of the adrenal glands and the body weights in the second half of gestation but they reported only one peak at 35 days. These authors weighed both glands. The difference in this study may be due to a difference in growth rate of the right adrenal gland compared to the left. The right gland is surrounded by the liver and its growth may thus be restricted. McNulty et al, (1981) reported that the left adrenal is heavier than the right. In the fetal rat a fall in relative adrenal weight in the last 3 to 4 days of gestation was observed by Cohen (1976) and by Fullerton (1983). While the relative adrenal weight in fetal pigs increased in the last days of gestation (Dvorak, 1972) as it does in fetal monkeys (Kerr et al, 1969).

Neonatal

After birth the absolute weight of the left adrenal gland showed a steady increase in the neonatal guinea pig between day 2 and

20 (Fig. R1-14). The same observation was reported by Dalle and Delost, (1974). Also the relative weight of the left adrenal gland was significantly increased after birth to reach a maximum value at day 4 (Fig. R1-15). In pigs the absolute and relative weights of the adrenal gland reach a maximum value on the day of birth and remain high until day 10 (Dovorak, 1972). In sheep the absolute weight of the adrenal gland decreases after birth and increases at day three (Madill and Bassett, 1973). In the human and monkey the absolute weight decreases by one third in the first two weeks after birth (McNulty et al, 1981). In the rat the relative adrenal weight falls rapidly after birth to reach a minimum value by day six (Fullerton, 1983). Doering and Clayton (1969) suggested that when the adrenal glands are small they secrete less hormone than when they are larger. This study suggests that the fall in relative adrenal weight is associated with decreased ability to synthesise steroid in fetal guinea pigs. After birth the increase in the relative weight of the adrenal gland may be an important factor in the increased plasma cortisol seen after delivery until 21 days post partum. Also the present results indicate that the adrenal cortex of newborn guinea pigs has a high corticosteroid production capacity which is enhanced by ACTH stimulation. The fall in the relative weight after six days may be explained by an earlier fall in ACTH secretion as a result of the suppression from the large amounts of cortisol secreted after the stresses of delivery and cold exposure.

Adrenal Histology

The results of the present study confirm those of Black (1972) who found the adrenal cortex of fetal guinea pigs at 30 days consisted of two zones which appear to correspond to the outer zona glomerulosa and inner zona fasciculata of the mature gland. At 30 days of gestation the adrenal cortex of fetal guinea pigs is large (Fig. R2-8). In fetal guinea pigs, man, rat, rabbit and cat the adrenal gland appears first as a mass of differentiating cells and these differentiating parencymal cells are loosely organised. The organ is poorly vascularised (Robinson, Rowe and Wintour, 1979b). In fetal lambs before 100 days of gestation two zones were reported by Alexandra, Britton, James, Nixon, Parker, Wintour and Wright (1968) and by Webb (1980).

From 35 to 50 day the cells increase in size and number. The greatest increase in the thickness of the zona fasciculata occurs between day 50 and 60 and the cell cords become straight as in the adult. A similar observation was reported by Black (1972). This author also described the zona fasciculata cells as having abundant lipid like those of the adult and said that the differentiation of the inner zone into zona reticularis and fasciculata occurred at 55 days. The difference between this study and that of Black in the timing of the differentiation of the inner zone probably relates to differences in the staining procedures used. Black (1972) used paraphenylenedimine stain while in this study M&H stain, and haematoxylin and eosin were used. The boundary between the zona fasciculata and the zona reticularis is not clear until day 64 with heamatoxylin and eosin stain. In sheep the inner zone cells remain relatively immature before 100 days (Robinson et al, 1979) and at 100 days of gestation a third fetal zone was reported by Nathanielsz et al, (1972).

From histological observations and physical measurements the present study indicates that the adrenal cortex grows faster from day 45 to day 60 and the majority of this terminal growth takes place in the zona fasciculata. During this period cell number in the zona fasciculata

increases. Individual cells grow larger with increasing amount of complex smooth endoplasmic reticulum and mitochondria (Black, 1972). In fetal sheep an increase of about 5 to 6 fold occurs in the size of the adrenal cortex in the last 8 days of intrauterine life (Nathanielsz et al, 1977). Durand et al, (1979) also reported that the greatest increase in ovine fetal adrenal cell size and number occurred between 136 and 144 days of gestation. In pigs this increase occurs between day 105 and 113 (Lohes and First, 1981). In humans the two zones of the fetal adrenal cortex, the fetal zone which comprises about 80 % of the fetal adrenal cortex at term and the adult type cortical zone develop separately from each other during fetal life (Johannison, 1968).

Robinson et al, (1979) described the ultrastructural development of the fetal sheep adrenal. The increase in smooth endoplasmic reticulum and mitochondrial membrane occurs between 120 days and term. In humans the ultrastructural changes occur during the second trimester and increase during the third trimester (Johannison, 1968). In the guinea pig large areas of the complex smooth endoplasmic reticulum with mitochondria appear in late fetal life (Black, 1972). This histological study has shown that during fetal life the fetal adrenal cortex enlarged steadily. Growth of the adrenal cortex results largely from hypertrophy of the zona fasciculata cells.

Dexamethasone Treatment

This is the first study of the effects of dexamethasone treatment on plasma hormone levels in the pregnant and fetal guinea pig. The decrease in fetal body weight and organ weights observed in this study are supported by the observations of Beck and Johnson (1980) and Garvey, Migally, Sullivan, and Sullivan (1983). They studied the effects of dexamethasone in species having short gestation (mice, rats and rabbits) and documented both general body and specific organ growth inhibition. McNulty et al, (1981) and Novy and Walsh (1983) administered dexamethasone to pregnant monkeys for several weeks and reported retarded fetal growth and decreased adrenal weights. Glucocorticoid treatment at such dose levels reduces placental weight (Table R7-1) and may interfere with placental function. This was also reported by Beck and Johnson (1980) and poses significant risks for the fetuses. In adult male guinea pigs prolonged dexamethasone treatment has no effects on the body weight (Obara, Mikami, Stortt, 1984).

A decrease in adrenal weight, adrenal cortisol concentration and serum cortisol was reported in the adult male guinea pig treated with dexamethasone (Obara et al, 1984). A decrease in maternal and fetal plasma and adrenal cortisol has also been reported in other species. In the cow Comline et al, (1974) and Hoffmann, Schams, Gimenez, Ender, Lerrmann, and Kary (1973) reported that maternal dexamethasone depressed both maternal and fetal cortisol concentrations. In monkeys administration of dexamethasone for several weeks decreased basal levels of maternal cortisol (McNulty et al, 1981). Dexamethasone treatment of pregnant women during the third trimester causes a decreased cortisol concentration (Brown, Strott and Liddle, 1972).

There is abundant evidence that glucocorticoids can effect the secretion of several pituitary hormones in addition to ACTH and including TSH (Pamenter and Hedge, 1980) and that they can inhibit the hypothalamus-pituitary-adrenal system (D'Angelo, Paul and Wall,

1973). This interference with the adrenotrophic influence is manifested in the present study by decreased fetal adrenal weight and a decrease in adrenal gland cortisol concentration. It is probable that fetal adrenal cortisol release is depressed by the high circulating levels of exogenous dexamethasone. Thus the lower fetal cortisol values measured in this study are likely to be largely of maternal origin. The rate of transfer of cortisol across the placenta increases with gestational age (Dalle and Delost, 1979). The high level of CBG and the low MCR in the fetal guinea pig (Delost et al, 1983) and the stress of dexamethasone injection could play a role in the maintenance of the high levels of cortisol in the maternal and fetal guinea pigs.

There was a decrease in the plasma T4 and T3 of the maternal and fetal guinea pig (Table R7-2) after dexamethasone treatment. A reduction in serum T4 and T3 was also reported in control human subjects and patients after dexamethasone treatment (Degroot and Hoye, 1976; Burr, Ramsden, Griffiths and Black, 1976; Duick, Warren, Nicoloff, Otis and Croxson, 1974) as well as in patients with Grave's disease (Chopra et al, 1975a). These workers suggested that the effects of dexamethasone treatment were partly mediated by depression of pituitary TSH and hence thyroid hormone secretion. An increase in the rT3 after dexamethasone treatment was reported by Chopra et al, (1975a) and by Burr et al, (1976). They found the increase in the serum rT3 on the first day of dexamethasone treatment to be similar to the fall in T3 and suggested that peripheral metabolism of T4 is redirected to produce rT3 rather than T3. A later fall in T4 and T3 may be attributed to decreased plasma TBG concentrations.

In the fetal sheep continuous infusion of cortisol to the fetus resulted in a fall in fetal plasma T4 concentration and an increse in the fetal T3 concentration (Thomas et al, 1978). In the fetal calf administration of cortisol to the fetus resulted in a fall in plasma T4 and TSH (Thomas et al, 1974). Fetal plasma T3 may be derived from two sources (i) secretion by the fetal thyroid and (ii) T3 release from peripheral tissues after monodeiodination of T4. It

appears that in the fetus cortisol is able to increase plasma T3 concentrations by increasing the rate of production from T4 in the tissues (Thomas et al, 1978).

The data of the present study suggest that the effects of dexamethasone on plasma throxine are shared by the endogenous glucocorticoid. It is possible that the high levels of glucocorticoids could effect the secretion and metabolism of the maternal and fetal thyroid leading to reduction in circulating T4 and T3 levels near term. These effects may be the result of (i) changes in the activity of liver T4 monodeiodinase (ii) influences on the hypothalamus pituitary thyroid system to inhibit TSH production and so reduce thyroxine synthesis and secretion from the thyroid gland (iii) direct effects on the thyroid gland. In the fetuses the T3/T4 ratio increased significantly compared with normal fetal guinea pigs of the same gestational age (Table R7-3).. This means that there is a greater T3 production from the reduced T4 level. In fetal sheep an increase in plasma glucocorticoids leads to increased <u>in vitro</u> deiodination of T4 to T3 in the liver (Wu et al, 1977). The decrease in the plasma T3 level in fetal guinea pigs following dexamethasone treatment is proportionately less than the decrease seen in T4 suggesting that a similar effect of glucocorticoids on peripheral deiodination is occurring.

The decrease in the % free T4 (Table R7-4) could be a result of the decrease in plasma T4 concentration or an increase in the concentration of TBPA which would reduce the proportion of free hormone (Burr et al, 1976).

The failure of high doses of dexamethasone to induce parturition when administered to the mother agrees with the observation of Illingworth et al, (1974) and Donovan and Peddie (1974) who showed that parturition occurs at the normal time after dexamethasone treatment. It appears that fetal cortisol is not a trigger to parturition in the guinea pig.

Maternal Thyroidectomy

No reports are available in the literature for the effects of thyroidectomy in guinea pigs during pregnancy. The maintenace of pregnancy in the guinea pigs after maternal surgical thyroidectomy did not appear to be affected by the maternal hypothyroid state. The fetuses were slightly lighter. The fetal body weight appeared, to some extent, to be affected by the significant decrease in placental size (Table R9:1). Thus maternal thyroid hormones appear to play a role in placental and fetal development during gestation. Potter et al, (1986) have reported similar affects in sheep after maternal thyroidectomy. In monkeys, changes have been reported by Holt, Cheek and Kerr (1973) as a result of maternal and fetal hypothyroidism following radioiodine (2 µCi/Kg) intravenously at 71-88 days of gestation.

It is likely that the deficiency of thyroid hormones of maternal origin could play a role in fetal thyroid and pituitary development, as judged by measurment of weight and by histological studies. The histological changes in both these glands and the increase in T4 in the fetal thyroid glands (Table R9-2) indicated that these glands were active and therefore the results presented here agree with the previous suggestion that the fetal pituitary-thyroid system is functioning at a relatively high level. As shown in Table R9-2, there was a significant difference between the T4 levels in the plasma of thyroidectomised guinea pigs and their fetuses compared with 60 day normal animals. Similar data were reported in sheep after maternal thyroidectomy (Potter et al, 1986) and in the monkey (Holt et al, 1973).

Structural changes in the guinea pig placenta have been studied by Kaufmann and Davidoff (1977). Relevant to the present discussion is the observation that most of the placental mass increase during pregnancy can be attributed to the exchange area which expanded until full term. This expansion has been shown to occur by vascularisation of the interlobium and the transition zone. The latter area has been

shown to grow by trophoplast proliferation. That thyroid hormones cross the guinea pig placenta from the maternal to the fetal side has been suggested by the present work. Therefore the low level of fetal plasma T4 and T3 after maternal thyroidectomy could be due either to passage of the thyroid hormones from the fetus to the mother and/or to the active deiodination of T4 and T3 by placental inner ring deiodinases to yield T2 and T1 (Cooper et al, 1983 and Castro et al, 1985). T4 and T3 can cross the monkey placenta in either the maternal to fetal or fetal to maternal direction (Azukizawa et al, 1976).

The human and rat placenta contain relatively large amounts of iodothyronine tyrosyl deiodinase enzymes, which play a major part in the metabolism of T4 (Kaplan and Shaw, 1984). In the rabbit (Osorio, 1960) the human (Myant, 1958) and the rat (Wood, Sinha and Ekins, 1984; Obregon, Mallol, Pastor, Morreale de Escobar and Escobar del Rey, 1984) it has been reported that the placental transfer of T4 and T3 becomes more favourable during mid to late pregnancy.

There is a non-significant increase in plasma T3 concentrations (Table R9:2) in the plasma of thyroidectomised pregnant guinea pigs compared to the T3 level of 60 day normal pregnant guinea pigs. This increase could be due to the transfer of T3 from the fetus to the mother or it is possible that the guinea pig placenta contains an outer-ring deiodinase similar to that in the human and rat as reported by Kaplan and Shaw (1984). They found an outer-ring deiodinase in the placenta with activity greatest in the zone immediately adjacent to the uterine wall. Therefore iodothyronines that circulate in the fetal side of the placenta may have limited access to the outer ring deiodinase enzymes described by Kaplan and Shaw (1984). The activity of the β deiodinase 5':II in the cerebral cortex of hypothroid animals increased rapidly and markedly when serum T4 levels fell (Leonard, Silver, Kaplan, Mellen and Visser, 1984)

The low fetal adrenal gland cortisol concentrations (Table R9-2), can be explained by the increase in the activity of the fetal pituitary-thyroid system which results in increased basophil

cells rather than ACTH secreting cells. The fetal adrenal may also be suppresed by elevated maternal plasma cortisol concentrations resulting in a greater than normal contribution by the mother to fetal plasma cortisol levels.

The present data, plus the fact that guinea pigs are born very mature, support the concept of an autonomous development of the hypothalamic-pituitary-thyroid system in the guinea pig fetus. It suggests therefore, that the guinea pig can serve as a useful experimental model for development of this system in the human.

Further experiments which arise from this study might be :-

1- To study the placental transfer of T4 and T3 in the guinea pig at various gestational ages.

2- To investigate T3 secretion from the thyroid gland immediately after birth .

3- To study the effect of PTU treatment on neonatal development.

4- To study the effects of thyroidectomy on female fertility and fetal development.

REFERENCES

References

Adams, W.M. and Wagner, W.C. (1970).
The role of corticoids in parturition.
Biology of Reproduction 3, 223-228.

Alexandra, D.P., Britton, H.G., Cameron, E., Foster, C.L. and Nixon, D.A. (1973).
Adenophypophysis of fetal sheep: Correlation of ultrastructure with functional activity.
Journal of Physiology 230, 10-12P.

Alexandra, D.P., Britton, H.G., James, V.H.T., Nixon, D.A., Parker, R.A., Wintour, E.M. and Wright, R.D. (1968).
Steroid secretion by the adrenal gland of fetal and neonatal sheep
Journal of Endocrinology 40, 1-13.

Allen, J.P., Greer, M.A., McGilvra, R., Castro, A. and Fisher, D.A. (1974).
Endocrine function in an anencephalic infant.
Journal of Clinical Endocrinology and Metabolism 38, 94-98.

Anderson, D.C. and Winter, J.S.D. (1985).
The adrenal cortex in the fetus and neonate.
In:- The Adrenal Cortex in The Fetus and Neonate.
Pub. Butterworth & Co. Ltd. pp 32-56.

Anderson, D.F., Stock, M.K. and Rankin, J.H.G. (1979).
Placental transfer of dexamethasone in near-term sheep.
Journal of Developmental Physiology 1, 431-436.

Avery, M.E. (1975).
Pharmacological approaches to the acceleration of fetal lung maturation.
British Medical Bulletin 31, 13-17.

Azarnoff, D.L. and Leathem, J.H. (1948).
Propylthiouracil action in the guinea pig.
Anatomical Record 101, 722.

Azukizawa, M., Murata, Y., Ikenoue, T., Martin, J.R.C.B. and Hershman, J.M (1976).
Effect of Thyrotropin-Releasing Hormone on secretion of thyrotropin, prolactin, T4 and T3 in pregnant and fetal rhesus monkeys.
Journal of Clinical Endocrinology and Metabolism 43, 1020-1028.

Balfour, W.E. (1969).
Inhibition of thyrotropin secretion and deiodination of T4.
Journal of Physiology 200, 48-49P.

Ballard, P. L., Baxter, J.D., Higgins, S.L., Rousseau, G.G. and Tomkins, G.K. (1974).
General presence of glucocorticoid receptors in mammalian tissues.
Endocrinology 94, 998-1002.

Barr, A., Lugg, M.A. and Nicholas, T.A. (1980).
Cortisone and cortisol in maternal and fetal blood and in amniotic fluid during the final ten days of gestation in the rabbit.
Biology of the Neonate 38, 214-220.

Bassett, J.M. and Alexander, G. (1971).
Insulin, growth hormone and corticosteroids in neonatal lambs, normal concentrations and the effects of cold.
Boilogy of Neonate 17, 112-125.

Bassett, J.M. and Thorburn, G.D. (1969).
Fetal plasma corticosteroids and the initiation of parturition in sheep.
Journal of Endocrinology 44, 285-286.

Baudry, M., Clot, J.P., Bouhnik, J., Michel, O. and Michel, R. (1975).
Thyroid hormone action on electrophoresis patterns of proteins of the rat liver and muscle mitochondria.
Seventh International Thyroid Conference, Boston, Mass, Abstract 118.

Beck, J.C. and Johnson, J.W.C. (1980).
Maternal adminstration of glucocorticoids.
Clinical Obstetric and Gynecology 12, 93-113.

Becks, H., Scow, R.O., Simpson, M.E., Asling, C.W., Li, C.H. and Evans, H. M. (1950).
Response by the rat thyro-parathyroidectomised at birth to growth hormone and to thyroxine given separately or in combination
Anatomical Record 107, 299-317.

Beitins, I.Z., Kowarski, A., Shermata, D.W., Delemos, R.A. and Migeon, C. (1970).
Fetal and maternal secretion rate of cortisol in sheep : diffusion resistance of the placenta.
Pediatric Research 4, 199-134.

Benircshke, K. (1956).
Adrenals in anencephaly and hydrocephaly.
Obstetrics and Gynecology 8, 412-425.

Black, V. H. (1972).
The developmenet of smooth-surfaced endoplasmic reticulum in adrenal cortical cells of fetal guinea pigs.
American Journal of Anatomy 135, 381-418.

Bloch, E. (1969).
The metabolism of pregnenolone and progesterone by adrenal homogenates of fetal guinea pigs and other mammalian fetuses.
Steroids 13, 589-605.

Bloch, E., Benirschke, K. and Rosemberg, E. (1956).
C19 steroid, cortisol and a sodium retaining factor in human fetal adrenal glands.
Endocrinology 58, 626-633.

Bongiovanni, A.M., Ederlein, W.R., Westphal, M. and Boggs, T. (1958).
Prolonged turnover rate of hydrocortisone in the new-born infant.
Journal of Clinical Endocrinology and Metabolism 18, 1127-1130.

Boshier, D.P., Halloway, H. and Liggins, G.C. (1980).
Growth and cytodifferentiation of the fetal lamb adrenal cortex prior to parturition.
Journal of Anatomy 130, 97-111.

Bressler, R. and Wittels, B. (1966).
The effect of T4 on lipid and carbohydrate metabolism in the heart
Journal of Clinical Investigation 45, 1326-1333.

Brewer, D.B. (1957).
Congenital absence of the pituitary gland and its consequences.
Journal of Pathology and Bacteriology 73, 59-67.

Brown, R.D., Strott, C.A. and Liddle, G.W. (1972).
Plasma deoxycorticosterone.
Journal of Clinical Endocrinology and Metabolism 36, 736-742.

Bruce, N.W. and Abdul-Karim, R.W. (1973).
Relationships between fetal weight, placental weight and maternal placental circulation in the rabbit at different stages of gestation.
Journal of Reproduction and Fertility 32, 15-24.

Bruck, K. (1983).
Functions of the endocrine system .
In :- Human Physiology By :- Schmidt, R.F. and Thews, G.
(Pub. Springer) pp 658-687.

Brzezinka-Slebodzinska, E. and Slebodzinki, A.B. (1986).
Simultaneous observations of iodothyronine content in the thyroid gland, plasma and T4- 5' and 5-monodeiodinase activity in liver, during the neonatal period of the pig.
Journal of Developmental Physiology 8, 79-86.

Buchanan, J.L., Primack, M.T. and Tapley, D.F. (1971).
Effect of inhibition of mitochondrial protein synthesis in vitro upon stimulation of oxygen consumption.
Endocrinology 89, 534-537.

Burman, K.D., Read, J., Dimond, R.C., Strum, D., Wright, F.D., Patow, W., Earll, J.M. and Wartonfsky, L. (1976).
Measurment of rT3, T3 and T4 in human amniotic fluid and in cord and maternal serum.
Journal of Clinical Endocrinology and Metabolism 43, 1351-1359.

Burr, W.A., Ramsden, D.B., Griffiths, R.S. and Black, E.G. (1976).
Effect of a single dose of dexamethasone on serum concentrations of thyroid hormones.
Lancet 2, 58-61.

Cabello, C. and Levieux, D. (1980).
Neonatal changes in the concentrations of thyrotrophin, T4, T3 and cortisol in the plasma of preterm and full term lambs.
Journal of Developmental Physiology 2, 59-69.

Cason, J.E. (1950).
A rapid, one-step Mallory-Heidenhain stain for connective tissue.
Stain Technology 25, 225-226.

Castro, M.I., Alex, S., Young, R.R., Braverman, L.E. and Emerson, C.H. (1986).
Total and free serum thyroid hormone concentrations in fetal and adult pregnant and non pregnant guinea pigs.
Endocrinology 118, 533-537.

Castro, M.I., Braverman, L.E., Alex, S., Wu, C.F. and Emerson, C.H. (1985).
Inner ring deiodination of T3 in the in situ perfused guinea pig placenta.
Journal of Clinical Investigation 76, 1921-1926.

Cavalieri, R.G., Castle, J.N. and Mcmahon, F.A. (1984)
Effects of dexamethasone on kinetics and distribution of T3 in the rat.
Endocrinology 114, 215-221.

Chopra, I.J., Sack, J. and Fisher, D.A. (1975).
rT3 and T3 in fetal and adult sheep: studies of MCR production rates serum binding and thyroidal content relative to T4.
Endocrinology 97, 1080-1088.

Chopra, I.J., Williams, D.E., Orgiazzi, J. and Solomon, D. (1975a)
Opposite effects of dexamethasone on serum concentrations of rT3 and T3.
Journal of Clinical Endocrinology and Metabolism 41, 911-920.

Cohen, A. (1976).
Adrenal and plasma B levels in the pregnant, fetal and neonatal rat, in the perinatal period.
Hormone and Metabolic Research 8, 474-478.

Colenbrander, B., MacDonald, A.A., Wong, C.C. and Parvizi, N. (1980).
Plasma concentrations of thyroid-stimulating hormone, T4, T3 in the perinatal pig.
Journal of Endocrinology 85, 38P.

Comline, R.S., Hall, L.W., Lavelle, R.S., Nathanielsz, P.W. and Silver, M. (1974).
Parturition in the cow: endocrine changes in animals with chronically implanted catheters in the fetal and maternal circulations
Journal of Endocrinology 63, 451-472.

Comline, R.S. and Silver, M. (1961).
The release of adrenaline and noradrenaline from the adrenal glands of the fetal sheep.
Journal of Physiology 156, 424-444.

Cooper, E., Gibbens, M., Thomas, C.R. and Lowy, C. (1983).
Conversion of T4 to rT3 in the guinea pig placenta: in vivo studies.
Endocrinology 112, 1808-1815.

Currie, W.B. and Thorburn, G.B. (1977).
Parturition in goats: studies on the interactions between the fetus, placenta, prostaglandin F and progesterone before parturition, at term or at parturition induced prematurely by corticotrophin infusion of the fetus.
Journal of Endocrinology 73, 263-278.

Dalle, M. and Delost, P. (1974).
Changes in the concentrations of cortisol and corticosterone in the plasma and adrenal glands of the guinea-pig from birth to weaning.
Journal of Endocrinology 63, 483-488.

Dalle, M. and Delost, P. (1976).
Plasma and adrenal cortisol concentrations in fetal, newborn and mother guinea pigs during the perinatal period.
Journal of Endocrinology 70, 207-214.

Dalle, M. and Delost, P. (1979).
Fetal-Maternal production and transfer of cortisol during the last days of gestation in the guinea-pig.
Journal of Endocrinology 82, 43-51.

Dalle, M., Pradier, P. and Delost, P. (1983).
The conversion of cortisol into its principal metabolites, their tissular concentrations and transplacental transfer during cortisol infusion of mother and fetal guinea pigs.
Steroids 42, 511-523.

D'Angelo, S.A. (1966a).
A comparative study of TSH and FSH secretion in rat and guinea pig : effects of gonadectomy and goitrogens.
Endocrinology 78, 1230-1237.

D'Angelo, S.A. (1966b).
Thyro-adrenal relationships in maternal fetal and neonatal guinea pigs. Effects of goitrogens.
Proceeding of The Society For Experimental Biology and Medicine 121, 555-559.

D'Angelo, S. A. (1967).
Pituitary-thyroid interrelations in maternal, fetal and neonatal guinea pigs.
Endocrinology 81, 132-138.

D'Angelo, S.A., Paul, D.H. and Wall, N.R. (1973).
Maternal-fetal endocrine interrelations: influence of maternal adrenocorticosteroids on fetal ACTH secretion.
American Journal of Physiology 244, 543-547.

D'Angelo, S.D., Stevens, C.E., Paschkis, K.E. and Cantarow, A.(1953).
Influence of cortisone on thyroid-pituitary interaction in normal and goitrous rats.
Proceedings of Society For Experimental Biology and Medicine 83, 181-183.

Davis, M.E. and Plotz, E.J. (1954).
The effects of cortisone acetate on intact and adrenalectomized rats during pregnancy.
Endocrinology 54, 384-395.

Degroot, L.J. and Hoye, K. (1976).
Dexamethasone suppression of serum T3 and T4.
Journal of Clinical Endocrinology and Metabolism 42, 976-978.

Diamond, M., Rust, N., and Westphal, U. (1969).
High-affinity binding of progesterone, testosterone and cortisol in normal and androgen-treated guinea pigs during various reproductive stages: relationships to masculinization.
Endocrinology 84, 1143-1151.

Doering, C.H. and Clayton, R.B. (1969).
Cholesterol side-chain cleavage activity in the adrenal gland of the young rat: development and responsiveness to ACTH.
Endocrinology 85, 500-511.

Donovan, B.T. and Peddie, M.J. (1973).
The development of the hypophysial portal system in the guinea pig
Journal of Anatomy 114, 292p.

Donovan, B.T. and Peddie, M.J. (1974).
Adrenal function oestrogen and the control of parturition in the guinea pig.
Journal of Endocrinology 61, xxi.

Draper, R.L. (1920).
The prenatal growth of the guinea pig.
Anatomical Record 18, 369-392.

Duick, D.S., Warren, D.W., Nicoloff, J.T., Otis, C.L. and Croxson, M.S. (1974).
Effect of single dose dexamethasone on the concentration of serum T3 in man.
Journal of Clinical Endocrinology and Metabolism 39, 1151-1154.

Durand, P. (1979).
ACTH receptor levels in lamb adrenal at late gestation and early neonatal stages.
Biology of Reproduction 20, 837-845.

Dussault, J.H., Hobel, G.T. and Fisher, D.A. (1971).
Maternal and fetal thyroxine secretion during pregnancy in the sheep.
Endocrinology 88, 47-51.

Dvorak, M. (1972).
Adrenocortical function in fetal, neonatal and young pigs.
Journal of Endocrinology 54, 473-481.

Eayrs, J.T. (1960).
Influence of the thyroid on the central nervous system.
British Medical Bulletin 16, 122-127.

Eckstein, P., Mckeown, T. and Record, P.G. (1955).
Variation in placental weight according to litter size in the guinea-pig.
Journal of Endocrinology 12, 108-114.

Edelhoch, H. and Robbins, J. (1978).
"Thyroglobulin Chemistry and Biosynthesis" .
In:-The Thyroid (eds. Werner, S.G. and Ingbar, S.H.) pp 62-76.

Edelman, I.S. and Ismail-Beigi, F. (1974).
Thyroid thermogenesis and active sodium transport.
Recent Progress in Hormone Research 30, 235-257.

Eguchi, Y. (1961).
Atrophy of the fetal mouse adrenal following decapitation in utero.
Endocrinology 68, 716-718.

Eguchi, Y., Fukiishi, Y. and Hasegawa, Y. (1980).
Ontogeny of the pituitary-thyroid system in fetal rats: observations on the fetal thyroid after maternal treatment with goitrogen.
Anatomical Record 198, 637-642 .

Eguchi, Y., Suzuki, S., Morikawa, Y. and Hashimoto, Y. (1971).
Experimantal formation of goiter in exencephalic fetal rats subjected to maternal hypervitaminosis A.
Endocrinology 88, 261-263.

Elgee, N.J. and Williams, R.H. (1955).
Efects of thyroid function on insulin degradation.
Amercian Journal of Physiology 180, 13-20.

Elvidge, H. (1972).
Production of dated pregnant guinea pigs without post partum matings.
Journal of The Institute of Animal Technicians 23, 111-117.

Erenberg, A., Rhodes, M.L., Weinstein, M.M. and Kennedy, R.L.(1979)
The effect of fetal thyroidectomy on ovine fetal lung maturation.
Pediatric Research 13, 230-235.

Etta, K.M. (1973).
"Comparative studies of the relationships between serum thyroxine and thyroxine binding-globulin".
Ph.D. Thesis, Michigan-State University, 1971.

Fahmy, D., Read, G.F., and Hillier, S.G. (1975).
Some observations on the determination of cortisol in human plasma by radioimmunoassay using antisera against cortisol-3-BSA.
Steroid 26, 267-280.

Fajer, A.A. and Vogt, M. (1963).
Adrenocortical secretion in the guinea-pig.
Journal of Physiology 169, 373-385.

Fentener Van-Vlissinqen, J.M., Colenbrander, B. and Wensing, C.J.G. (1983).
Morphological development of the thyroid gland and serum T4 concentration in the intact and decapitated pig fetus.
Journal of Developmental Physiology 5, 361-371.

Firth, J.A. and Farr, A. (1977).
Structural features and quantitative age-dependent changes in the inter-vascular barrier of guinea pig haemochorial placenta.
Cell and Tissue Research 184, 507-517.

Fisher, D.A. and Dussault, J.H. (1975).
"The Thyroid" In:- American Handbook of Physiology, Section 7, Endocrinology III pp 21-38.

Fisher, D.A., Dussault, J.H., Erenberg, A. and Lam, R.W. (1972).
T4 and T3 metabolism in maternal and fetal sheep.
Pediatric Research 6, 894-899.

Fisher, D.A., Dussault, J.H., Hobel, C.J. and Lam, R.W.(1973).
Serum and thyroid gland T3 in human fetus.
Journal of Clinical Endocrinology and Metabolism 36, 397-400.

Fisher, D.A., Dussault, J.H., Sack, J. and Chopra, I.J. (1977).
Ontogensis of hypothalamic-pituitary thyroid function and metaboloism in man, sheep and rat.
Recent Progress in Hormone Research 33, 59-107.

Fisher, D.A., Hobel, C.J., Garza, R. and Pierce, C.A. (1970).
Thyroid function in the preterm fetus.
Pediatrics 46, 208-216.

Fisher, D.A. and Odell, W.D. (1969).
Acute release of thyrotrophin the newborn.
Journal of Clinical Investigation 48, 1670-1677.

Fisher, D.A., Oddie, T.H. and Makoski, E.J. (1966).
The influence of environmental temperature on thyroid adrenal and water metabolism of the new born infant .
Pediatrics 37, 583-591.

Fleischer, N. and Vale, W. (1968).
Inhibition of vasopressin-induced ACTH release from the pituitary by glucocorticoides in vitro.
Endocrinology 83, 1232-1236.

Flexner, L.B. (1955).
"Enzymatic and Functional Patterns of The Developing Mammalian Brain " In:- Biochemistry of The Developing Nervous Systm.
(ed. Waelsch, H.) Pub. Academic Press. pp 281-300.

Florsheim, W.H., Faircloth, M.A., Corcorran, N.L. and Rudko, P.(1966)
Prenatal thyroid function in the rat.
Acta Endocrinologica 52, 375-382.

Friedman, Y., Lang, M. and Burk, G. (1977).
Inhibition of thyroid adenlate cyclase by thyroid hormone: a possible locus for the short-loop negative feedback phenomenon.
Endocrinology 101, 858-868.

Fullerton, F.M. (1983).
"Thyroid Hormone and The Development of Adrenal Function in the Rat." Ph.D. Thesis. University of Southampton.

Funkhauser, J.D., Peevy, K.J., Mockride, P.W. and Hughes, E.R.(1978).
Distribution of dexamethasone between mother and fetus after maternal administration.
Pediatric 12, 1053-1056.

Gala, R.R. and Westphal, U. (1967).
Corticosteroid-binding activity in serum of mouse, rabbit and guinea pig during pregnancy and lactation: possible involvement in the initiation of lactation.
Acta Endocrinologica 55, 47-61.

Garvey, D., Migally, N., Sullivan, J. and Sullivan, L. (1983).
Suppression of adrenal cortical growth and differentiation in fetal rats exposed to dexamethasone.
Anatomical Record 205, 431-439.

Gillies, G., Linton, E.A. and Lowry, P. (1982).
Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin.
Nature 299, 355-356.

Glydon, R.St.J. (1957).
The development of the blood supply of the pituitary in the albino rat, with special reference to the portal vessel.
Journal of Anatomy 91, 237-244.

Gomez, C. L. (1971).
Hormonal influence on the biochemical differentiation of the rat cortex.
In "Hormones in development".
(M. Hamburgh and E. J. W. Barrington, eds) pp.417-435.
Appleton, New York.

Gray, B. and Gatton, U.A. (1974).
The transplacental passage of T4 and fetal thyroid function in the rat.
Acta Endocrinologica 75, 725-733.

Greer, M.A., Stott, K. and Milne, K.A. (1966).
Effect of thyiocyanate, perchlorate and other anions on thyroidal iodine metabolism.
Endocrinology 79, 237-247.

Greiner, J.W., Kramer, R.E. and Colby, H.D. (1976).
Changes in adrenocortical function in male and female guinea pigs during maturation.
Journal of Endocrinology 70, 127-134.

Gregerman, R.I. and Crowder, S.E. (1963).
Estimation of T4 secretion rate in the rat by the radioactive T4 turnover technique: influence of age, sex and exposure to cold.
Endocrinology 72, 382-392.

Griessen, M. and Lemarchend-Berand, T. (1973).
Thyrotropin secretion and metabolism in rats during PTU treatment
Endocrinology 92, 166-173.

Gross, J. and Pitt-Rivers, R. (1952).
Physiological activity of T3.
Lancet 262, 593-594.

Grumbach, M.M. and Werner, S.C. (1956).
Transfer of thyroid hormone across the human placenta at term.
Journal of Clinical Endocrinology 16, 1392-1394.

Hall, P.F. and Myant, N.B. (1956).
Passage of exogenous T4 and of iodide between mother and fetus
in pregnant rabbits.
Journal of Physiology 133, 181-193.

Haltmeyer, G.C., Denenberg, V.H., Thatcher, J. and Zarrow, M.M. (1966).
Response of the adrenal cortex of the neonatal rat after subjection
to stress.
Nature 212, 1373-1378.

Hamburg, M., Sobel, E.H., Koblin, R. and Rinestone, A. (1962).
Passage of thyroid hormones across the placenta in intact and
hypophysectomised rats.
Anatomical Record 144, 219-225.

Hani, A.E., Dalle, M. and Delost, D. (1980).
Sexual dimorphism in binding and metabolism of cortisol during
puperty in the guinea pig.
Journale de Physiologie (Paris) 76, 25-28.

Harris, G.W. (1948).
Neural control of the pituitary gland.
Physiology Reviews 28, 139-179.

Harris, H.A. (1936 & 1937).
The fetal growth of the sheep.
Journal of Anatomy 54, 516-527.

Harrison, F.A. and Patterson, J.Y.F. (1980).
"The Adrenal Cortex".
In :- Scientific Foundations of Veterinary Medicine.
(eds :- Phillipson, A.T., Hall, L.W. and Pritchard, W.R.)
Pub. Heinemann Medical Books. pp 26-32.

Haugen, H.N., Brinck-Johnsen, T. and Knutrud, O. (1967).
The adrenocortical function in the first week of life.
Zeits Kinderchir Fuer Grenzgebiet 5, 149-156.

Herd, P. Kaplay, S.S. and Sanadi, D.R. (1974).
On the origin and metabolism of action of T4 responsive protein.
Endocrinoogy 94, 464-474.

Hill, J.R. (1958).
The relation between oxygen consumption, hypoxia and environmental temperature.
Journal of Physiology 143, 64P.

Hillier, A.P. (1968).
Thyroxine deiodination during cold exposure in the rat.
Journal of Physiology 197, 135-147.

Hoar, R.M. (1965).
The normal, maternal and fetal peripheral blood plasma and adrenal crotical levels of hydrocortisone throughout pregnancy in guinea pigs.
Anatomical Record 151, 362.

Hoffmann, B., Schams, D., Gimenez, T., Ender, M.L., Lerrmann, G. and Karg, H. (1973).
Changes of progesterone, total oestrogens, corticosteroids, prolactin and LH in bovine peripheral plasma around parturition with special reference to the effect of exogenous corticoids and a prolactin inhibitor respectively.
Acta Endocriologica 73, 385-395.

Holt, A. B., Cheek, D. B. and Kerr, G. R. (1973).
Prenatal hypothyroidism and brain composition in a primate.
Nature 243, 413-414.

Hopkins, P.S. and Thorburn, G.D. (1971).
Placental permeability to maternal T4 in the sheep.
Journal of Endocrinology 49, 549-550.

Hopkins, P.S. and Thorburn, G.D. (1972).
The effects of fetal thyroidectomy on the development of the ovine fetus.
Journal of Endocrinology 54, 55-66.

Hoskins, L.C., Vanarsdel, P.L. and Willams, R.H. (1958).
Placenta transmission and mammary gland secretion of T4 in the rat.
Amercian Journal of Physiology 193, 509-515.

Hull, D. (1966).
The structural and function of brown adipose tissue.
British Medical Bulletin 22, 92-96.

Humason, G.L. (1972).
"Staining connective tissue and muscle".
In:- Animal Tissue Techniques.
(ed. G.L. Humason). Chapter 13 pp. 173-199.

Ibsen, H.L. (1928).
Prenatal growth in guinea pigs with special reference to environmental factors affecting weight at birth.
Journal of Experimental Zoology 51, 51-91.

Iino, S.T., Yamada, T. and Greer, M.A. (1961).
Effect of graded doses of PTU on biosynthesis of thyroid hormones.
Endocrinology 68, 582-588.

Illingworth, D.V., Challis, J.R.G., Ackland, N., Burton, A.M., Heap, R.B. and Perry, J.S. (1974).
Parturition in the guinea pig; plasma levels of steroid hormones steroid-binding proteins, and oxytocin, and the effect of corticosteroids, prostaglandins and adrenocorticotrophin.
Journal of Endocrinology 63, 557-570.

Imura, H., Sparks, L.L., Grodsky, G.M. and Forsham, P.H. (1965).
Immunologic studies of ACTH. Dissociation of biologic and immunologic activities.
Journal of Clinical Endocrinology and Metabolism 25, 1361-1369.

Irvine, C.H.G. and Evans, M.J. (1975).
Postnatal changes in total and FT4 and T3 in foal serum.
Journal of Reproduction and Fertility, Supplement 23, 709-715.

Jacobson, A.G. and Brent, R.L. (1959).
Radioiodine concentration by the fetal mouse thyroid.
Endocrinolgy 65, 408-416.

Jansky, L. (1973).
Non-shivering thermogenesis and its thermo-regulatory significance
Biological Reviews 48, 85-132.

Johannisson, E. (1968).
The fetal adrenal cortex in the human. Its ultrastructure at different stages of development and in different functional states.
Acta Endocrinologica, Supplement 130.

Jones, C.T. (1974).
Corticosteroid concentrations in the plasma of fetal and maternal guinea pigs during gestation.
Endocrinology 95, 1129-1133.

Jones, C.T. (1976).
Lipid metabolism and mobilization in the guinea pig during pregnancy.
Chemistry Journal 156, 357-365.

Jones, C.T. (1985).
Reprogramming of metabolic development by restriction of fetal growth.
Biochemical Society Transactions 13, 89-91.

Jones, C.T., Lafeber, H.N. and Roebuck, M.M. (1985).
Studies on the growth of the fetal guinea pig changes in plasma hormone concentration during normal and abnormal growth.
Journal of Developmental Physiology 6, 461-472.

Jones, C.T. and Roebuck, M.M. (1980).
The development of the pituitary-adrenal axis in the guinea pig.
Journal of Steroid Biochemistry 12, 77-82.

Jones, C.T. and Roebuck, M.M. (1980a).
ACTH peptides and the development of the fetal adrenal.
Acta Endocrinologica 94, 107-116.

Josimovich, J., Ladman, A.J. and Deane, H.W. (1954).
A histophysiological study of the developing adrenal cortex of the rat during fetal and early postnatal stages.
Endocrinology 54, 627-639.

Jost, A. (1953).
Problems of fetal endocrinology: the gonadal and hypophyseal hormones.
Recent Progress in Hormone Research 8, 379-418.

Jost, A. (1966).
Problems of fetal endocrinology: the adrenal glands.
Recent Progress in Hormone Research 22, 541-571.

Jost, A. (1975).
"The Fetal Adrenal Cortex".
In:- The American Handbook of Physiology. Section 7,
Endocrinology pp 107-115.

Jost, A. and Picon, L. (1970).
Hormonal control of fetal development and metabolism.
Advances in Metabolic Disorders 4, 123-184.

Kaciuba-Uscilko, H. (1972).
Hormonal regulation of thermogenesis in the newborn pig.
Biology of the Neonate 21, 245-258.

Kahl, J., Wrenn, T.R. and Bitman, J. (1977).
Plasma T3 and T4 in young growing calves.
Journal of Endocrinology 73, 397-398.

Kamoun, A. (1970).
Activite cortico-surrenal au course de la gestation, de la
lactation et du development pre et postnatal chez le rat.
Journale de Physiologie (Paris) 62, 5-29.

Kaplan, M.M. and Shaw, E.A. (1984).
Type 11 iodothyronine 5' deiodination by human and rat
placenta in vitro.
Journal of Endocrionolgy and Metabolism 59, 253-259.

Kaufmann, P. and Davidoff, M. (1977).
The guinea-pig placenta.
Advances in Anatomy Embryology and Cell Biology 53, 1-99.

Kerr, G.R., Kennan, A.L., Waisman, H.A. and Allen, J.R. (1969).
Growth and development of the fetal rhesus monkey.
1- Physical growth.
Growth 33, 201-213.

Kerr, G.R., Tyson, I.B., Allen, J.R., Wallace, J.H. and Scheffler, G.(1972).
Deficiency of thyroid hormone and development of the fetal rhesus monkey. 1- Effect on physical growth, skeleton maturation and biochemical measures of thyroid function.
Biology of the Neonate 21, 282-295.

Kittinger, G.W. (1974).
Feto-maternal production and transfer of cortisol in the rhesus Macaca mulatta.
Steroids 23, 229-245.

Kittinger, G.W., Beamer, N.B., Hagemens, F., Hill, J.D., Banghman, W.L. and Ochsmer, A.J. (1972).
Evidence for autonomous pituitary-adrenal function in the near-term fetal rhesus "Macaca mulatta".
Endocrinology 91, 1037-1044.

Kirsch, R.E. (1938).
A study on the control of length of gestation in the rat with notes on maintenance and termination of gestation.
American Journal Physiology 122,86-93 .

Koneff, A.A., Nichols, C.W., Wolff, Jr.J. and Chaikoff, I.L. (1949).
The fetal bovine thyroid: morphogenesis as related to iodine accumulation.
Endocrinology 45, 242-249.

Krohn, P.L. and White, H.C. (1950).
The effect of hypothyroidism on reproduction in the female albino rat.
Journal of Endocrinology 6, 375-385.

Labrie, F., Raynaud, J. P., Ducommun, P., Fortier, C. (1964).
Influence of thyroid activity on corticosterone binding by plasma proteins.
Proceeding of Canadian Federal Biology Society 7, 44P.

Lamb, J.F., Ingran, C.G., Johnston, I.A. and Pitman, R.M.(1982).
"Endocrinology and metabolsim".
Essential Physiology (Pub Blackwells) pp 335.

Lascelles, A.K., and Setchell, B.P. (1959).
Hypothyroidism in the sheep.
Australian Journal of Biological Sciences 12, 455-465.

Lazo-wasem, E.A. (1960).
Pituitary ACTH levels during adrenal involution following thiouracil.
Proceeding of the Society for Experimental Biology and Medicine 103, 300-302.

Lee, Y., Liu, C.Y. and Hsu, H.T. (1970).
Effects of thyroid hormone on the guinea pig.
Endocrinology 86, 241-250.

Leonard, J.L., Silva, J.E., Kaplan, M.M., Mellen, S., Visser, T.J. and Larsen, P.R. (1984).
Acute posttranscriptional regulation of cerebrocortical and pituitary iodothyronine 5' deiodinases by thyroid hormone.
Endocrinology 114, 998-1004.

Leppaluoto, J., Huttunen, P. and Hirvonen, J. (1981).
The effect of deep hypothermia on the secretion of thyroid hormones and cortisol and oxygen consumption in guinea pigs.
Acta Physiologica Scandinavica 112, 417-420.

Liggins, G.C. (1968).
Premature parturition after infusion of corticotrophin or cortisol into fetal lambs.
Journal of Endocrinology 42, 323-329.

Liggins, G.C. (1976).
Adrenocortical-related maturational events in fetus .
American Journal of Obstetrics and Gynecology 126, 931-941.

Liggins, G.C., Fairclough, R.L., Grieves, S.A., Kendall, J.Z. and Knox, B.S. (1973).
The mechanism of initiation of parturition in the ewe.
Recent Progress in Hormone Research 29, 111-159.

Liggins, G.C. and Kennedy, P.C. (1968).
Effects of electrocoagulation of fetal lamb hypophysis on growth and development.
Journal of Endocrinology 40, 371-381.

Liggins, G.C., Kennedy, P.C. and Holm, L.W. (1967).
Failure of initiation of parturition after electrocoagulation of pituitary of the fetal lamb.
American Journal of Obstetrics and Gynecology 98, 1080-1086.

Loeb, J.N. (1978).
"Metabolic Changes." In :- "The Thyroid"
(eds Werners, S.G. and Ingbar, S.H.) pp 706.

Logothetopoulos, J. and Scott, R.F. (1956).
Histology and function of the developing fetal thyroid in normal and goitrous guinea pigs.
Journal of Endocrinology 14, 217-227.

Lohse, J.K. and First, N.L. (1981).
Development of the porcine fetal adrenal in late gestation.
Biology of Reproduction 25, 181-190.

Madill, D. and Bassett, J.M. (1973).
Corticosteroid release by adrenal tissue from fetal and newborn lamb in response to corticotrophin stimulation in a perfusion system *in vitro*.
Journal of Endocrinology 58, 75-87.

Malinowska, K.W., Hardy, R.N. and Nathanielsz, P.W. (1972a).
Plasma adrenocorticosteroid concentrations immediately after birth in the rat, rabbit and guinea pig.
Experientia 28, 1366-1367.

Malinowska, K.W., Hardy, R.N. and Nathanielsz, P.W. (1972b).
Neonatal adrenocortical function and its possible relation to the uptake of macromolecules by the small intestine of the guinea pig and rabbit.
Journal of Endocrinology 55, 397-404.

Malinowska, K.W. and Nathanielsz, P.W. (1974).
Plasma aldosterone, cortisol, corticosterone concentration in the newborn guinea pig.
Journal of Physiology 236, 83-93.

Mathur, H., Brown, B.L., Krane, E.J., Thomas, A.L. and Nathanielsz, P.W. (1980).
Thyroid hormone relationships in the fetal and newborn lamb.
Biology of the Neonate 37, 138-144.

Martin, K.O. and Black, V.H. (1982).
$^{4}\Delta$-Hydrogenase in guinea pig adrenal: evidence of localization in zona reticularis and age-related change.
Endocrinology 110, 1749-1757.

McIntosh, G.H., Potter, B.J., Mano, M.T., Hua, C.H. Cragg, B.G. and Hetzel, B.S. (1983).
The effect of maternal and fetal thyroidectomy on fetal brain development in the sheep.
Neuropathology and Applied Neurobiology 9, 215-223.

Mckeown, T. and Record, R.G. (1953).
The influence of placental size on fetal growth in man, with special reference to multiple pregnancy.
Journal of Endocrinology 25, 418-426.

McNulty, W., Novy, M.J. and Walsh, S.W. (1981).
Fetal and postnatal development of the adrenal glands in Macaca mulatta.
Biology of Reproduction 25, 1079-1089.

Mellor, D.J., Matheson, I.C., Small, J. and Wright, H. (1976).
Plasma T4 concentrations in ewes and their fetuses during the last six weeks of pregnancy.
Research in Veterinary Science 21 102-103.

Mitchell, B.F., Seron, F.M., Hess, D.L. and Jaffe, R.B. (1981).
Cortisol production and metabolism in the late gestation rhesus monkey fetus.
Endocrinology 108, 916-924.

Mitskevish, M.S. (1957).
In "Glands of Internal Secretion in The Embryonic Development of Birds and Mammals".
Academic Science of USSR. Moscow. Chapter 5, 104-151 p.

Mitskevich, M.S. (1962).
Hormonal interrelations between mother and fetus in mammals.
General and Comparative Endocrinology, Supplement 1, 300-308.

Moog, F. (1979).
Endocrine influence on the functional differentiation of the small intestine.
Journal of Animal Science 49, 239-249.

Moog, F. and Ortiz, E. (1960).
The functional differentiation of the small intestine.
VII.The duodenum of the fetal guinea-pig, with a note on the growth of the adrenals.
Journal of Endocrinology and Experimental Morphology 8, 182-197.

Moran-Campbell, E.J., Dickinson, C.T., Slater, J.D.H., Edwards, C.R.W. and Sikora, E.K. (1984).
"Clinical Physiology".
Pubs Blackwell Scientific Publications.

Mulay, S., Giannopoulos, G. and Solomon, S. (1973).
Corticosteroid levels in the mother and fetus of the rabbit during gestation.
Endocrinology 93, 1342-1348.

Murphy, B.E.P. (1979).
Cortisol and cortisone in human fetal development.
Journal of Steroid Biochemistry 11, 509-513.

Murphy, B.E.P. (1981).
Ontogeny of cortisol-cortisone interconversion in human tissues : a role for cortisone in human fetal development.
Journal of Steroid Biochemistry 14, 811-817.

Murphy, B.E.P. and Pattee, C.J. (1964).
Determination of T4 utilizing the property of protein-binding
Journal of Clinical Endocrinology and Metabolism 24, 187-196.

Myant, N.B. (1958).
The passage of T4 and T3 from mother to fetus in pregnant rabbits, with a note on the concentration of protein-bound iodine in fetal serum.
Journal of Physiology 142, 329-342.

Nathanielsz, P.W. (1969).
Peripheral utilization of T4 by the young growing calf.
Journal of Physiology 204, 43-45P.

Nathanielsz, P.W. (1969a).
Plasma T4 levels in the young lamb from birth to 61 days.
Journal of Endocrinology 45, 475-476.

Nathanielsz, P.W. (1970).
Thyroxine utilization in the newborn lamb.
Journal of Physiology 211, 157-171.

Nathanielsz, P.W. (1975).
Thyroid function in the fetus and newborn mammal.
British Medical Bulletin 31, 51-56.

Nathanielsz, P.W. (1976).
In "Fetal Physiology and Medicine".
(eds P.W. Nathanielsz and R. Beard) Pub Saunders, Philadelphia,
Pennsylvania. pp 215-231.

Nathanielsz, P.W., Abel, M. and Smith, G. W. (1973a).
In "Foetal and Neonatal Physiology", Sir Joseph Barcroft
Centenary Symposium. (eds R.S. Comline, K.W. Cross, G.S. Dawes.
and P.W. Nathanielsz) Pub Cambridge University Press. pp 594-602.

Nathanielsz, P.W., Comline, R.S., Silver, M. and Paisey, R.B. (1972).
Cortisol metabolism in the fetal and neonatal sheep.
Journal of Reproduction and Fertility, Supplement 16, 39-59.

Nathanielsz, P.W., Comline, R.S., Silver, M. and Thomas, A.L. (1973b).
Thyroid function in the fetal lamb during the last third of gestation.
Journal of Endocrinology 58, 535-546.

Nathanielsz, P.W., Jack, P.M.B., Krane, E.J., Thomas, A.L., Ratter, S. and Rees, L.H. (1977).
The role and regulation of corticotropin in the fetal sheep.
In "The Fetus and Birth ".
Ciba Foundation Symposium No. 47 (new series). Pub Elsevier Excerpta Medica North-Holland, (Amsterdam). pp 37-91.

Nathanielsz, P.W., Rossdale, P.D., Silver, M. and Comline, R.S. (1975).
Studies on fetal-maternal cortisol metabolism in the mare.
Journal of Reproduction and Fertility, Supplement 23, 625-638.

Nathanielsz, P.W., Silver, M. and Comline, R.S. (1973c).
Plasma T3 concentration in the fetal and newborn lamb.
Journal of Endocrinology 58, 683-684.

Nathanielsz, P.W. and Thomas, A.L. (1973).
Plasma T3 concentration in the newborn calf.
Experientia 29, 1426.

Newton, W.H. (1935).
"Pseudo-parturition" in the mouse, and the relation of the placenta to post-partum oestrus.
Journal of Physiology 84, 196-207.

Nishikawa, T. and Strott, C.A. (1984).
Cortisol production by cell isolated from the outer and inner zones of the adrenal cortex of the guinea pig.
Endocrinology 114, 486-491.

Novy, M.L. and Walsh, S.W. (1983).
Dexamethasone and estradiol treatment in pregnant rhesus macaques: effect on gestation length, maternal plasma hormones, and fetal growth.
American Journal of Obstetrics and Gynecology 145, 920-931.

Obara, T. Mikami, K. and Strott, C.A. (1984).
Differential suppression of the outer and inner zones of the adrenal cortex of the guinea pig.
Endocrinology 115, 1838-1841.

Obregon, M.J., Mallol, J., Pastor, R., Morreale de Escobar, G. and Escobar del Rey, F.(1984).
T4 and T3 in rat embryo before onset of fetal thyroid function.
Endocrinology 114, 305-307.

Oppenheimer, J.H., Squef, R., Surks, M.I. and Haver, H. (1963).
Binding of T4 by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in non-thyroidal illness.
Journal of Clinical Investigation 42, 1769-1782.

Ortiz, E. (1959).
The effect of PTU treatment of pregnant guinea pigs on the development of the fetus.
Anatomical Record 134, 620.

Osorio, C. (1960).
The passage of thyroid hormone from mother to fetus and its relation to fetal development.
British Medical Bulletin 16, 159-164.

Osorio, C. and Myant, N.B. (1962).
The binding of T4 by human fetal serum.
Clinical Science 23, 277-284.

Paisey, R.B. and Nathanielsz, P.W. (1971).
Plasma cortisol levels in the newborn lamb from birth to 30 days.
Journal of Endocrinology 50, 701-702.

Pals, A.J., Reineke, E.R. and Shaw, G.H. (1973).
Serum T4 levels in the perinatal guinea pig.
Laboratory Animal Science 23, 511-514.

Pamenter, R.W. and Hedge, G.A. (1980).
Inhibition of thyrotropin secretion by physiological levels of corticosterone.
Endocrinology 106, 162-166.

Pape, G.J. and Aibrecht, E.D. (1984).
Transutero-placental metabolism of cortisol and cortisone during mid and late gestation in the baboon.
Endocrinology 115, 1946-1951.

Pasqualini, J.R., Nguyen, B.L., Uhrich, F., Wiquist, N. and Diczfalusy, E. (1970).
Cortisol and cortisone metabolism in the human feto-placental unit at mid gestation.
Journal of Steroid Biochemistry 1, 209-219.

Pasternak, D.P., Socolow, E.L. and Ingbar, S.H. (1969).
Synergistic interaction of phenazone and iodide on thyroid hormone biosynthesis in the rat.
Endocrinology 84, 769-777.

Paterson, J.Y.F. (1973).
The rate constants for the interaction of cortisol and transcortin and the rate of dissociation of transcortin-bound cortisol in the liver.
Journal of Endocrinology 56, 551-570.

Paterson, J.Y.F. and Harrison, F.A. (1968).
The specific activity of plasma cortisol in sheep after intravenous infusion of cortisol, and its relation to the distribution of cortisol.
Journal of Endocrinology 40, 37-47.

Peterson, R.R. and Young, W.G. (1952).
The problem of placental permeability for thyrotrophin, PTU and T4 in the guinea pig.
Endocrinology 50, 218-225.

Pfannenstiel, P., Andrews, G.A. and Brown, D.W. (1965).
Congenital hypothyroidism from intra-uterine ^{131}I damage.
Current Topics in Thyroid Research, 749-758.

Pickering, D.E. (1968).
Thyroid physiology in the developing monkey fetus (Macaca Mulatta).
General and Comparative Endocrinology 10, 182-190.

Pickering, D.E. and Kontaxis, N.E. (1962).
Thyroid function in the fetus of the Macaque monkey (Macaca mulatta) 11. Chemical and morphological characteristics of the fetal thyroid gland.
Journal of Endocrinology 23, 267-275.

Pic, P. and Bouquin, J.P. (1985).
Thyrotropic hormone and thyroidal function initiation in fetal rat
Journal of Developmental Physiology 7, 207-214.

Platt, H. (1984).
Growth of the equine fetus.
Equine Veterinary Journal 16, 247-252.

Postel, S. (1957).
Placenta transfer of perchlorate and T3 in the guinea pig.
Endocrinology 60, 53-66.

Potter, P.J., McIntosh, G.H., Mano, M.T., Baghurst, P.A., Chavadej, J., Hua, C.H., Cragg, B.G. and Hetzel, B.S. (1986).
The effect of maternal thyroidectomy prior to conception on fetal brain development in sheep.
Acta Endocrinologica 112, 93-99.

Price, D. and Ortiz, E. (1965).
The role of fetal androgen in sex differentiation in mammals.
In :- "Organogenesis" .
(eds R.L.Dehaan and H. Ursprung) pp 629-652.

Rawlings, N. C. and Ward, W.R. (1978).
Effect of fetal hypophysectomy on the initiation of parturition in the goat.
Journal of Reproduction and Fertility 31, 249-254.

Refetoff, S. (1979).
Thyroid function tests.
In:- "Endocrinology" (ed. L.J. Degroot) pp 387-428.

Refetoff, S., Robin, N.I. and Fang, V.S. (1970).
Parameters of thyroid function in serum of 16 selected vertebrate species: a study of PBI, serum T4, FT4, and the pattern of T4 and T3 binding to serum proteins.
Endocrinology 86, 793-805.

Reichlin, S., Martin, J.B., Mitnick, M., Bosh, R., Grimm, Y., Bollinger, J., Gordon, J. and Malacura, J. (1972).
The hypothalamus in pituitary-thyroid regulation.
Recent Progress in Hormone Research 28, 229-286.

Riegle, G.D. and Nellar, J.E. (1967).
Changes in adrenal function during aging in cattle.
Journal of Gerontology 22, 83-87.

Riegle, G.D., Przekop, F and Neller, J.E. (1968).
Changes in adrenocortical responsiveness to ACTH infusion in aging goats.
Journal of Gerontology 23, 187-190.

Rivier, C., Vale, W. and Guilleman, R. (1973).
An *in vivo* CRF assay based on plasma levels of iodoimmunoassayble ACTH.
Proceedings of The Society of Experimental Biology and Medicine 142, 842-845.

Robinson, J.S., Kingston, J.E., Jones, C.T. and Thorburn, G.D. (1979). Studies on experimental growth retardation in sheep. The effect of removal of endometrial caruncles on fetal size and metabolism. Journal of Developmental Physiology 1, 379-398.

Robinson, P.M., Rowe, E.J. and Wintour, E.M. (1979). The histogenesis of the adrenal cortex in the fetal sheep. Acta Endocrinologica 91, 34-149.

Rosen, F. and Ezrin, C. (1966). Embryology of the thyrotroph . Journal of Clinical Investigation 26, 1343-1345.

Rosenthal, H.E., Slaunwhite, Jr.W.R. and Saudberg, A.A. (1969). Transcortin: a corticosteriod-binding protein of plasma. X. cortisol and progesterone interplay and unbound levels of these steroid in pregnancy. Journal of Clinical Endocrinology and Metabolism 29, 352-367.

Ross, T.B. (1967). Steroid synthesis in embryonic and fetal rat adrenal tissue. Endocrinology 81, 716-728.

Schonberger, W., Grimm, W., Emmrich, P. and Gempp, W. (1979). Thyroid administration lowers mortality in premature infant. The Lancet 2, 1181-1182.

Schwartz, H.L., Surks, M.T. and Oppenheimer, J.H. (1971). Quantition of extrathyroidal conversion of L-T4 to T3 in rat. Journal of Clinical Investigation 50, 1124-1130.

Selye, H., Collip, J.B. and Thompson, D.L. (1935). Endocrine interrelations during pregnancy. Endocrinology 19, 151-159.

Senga, O., Pittman, C.S., Lindsay, R.H, Chambers, J.B. and Hill, H.B. (1982).
Comparison of peripheral thyroid hormone metabolism in normal rats and in rats receiving prolonged glucagon infusion.
Endocrinology 110, 2011-2017.

Shepard, T.H., Andersen, H.J. and Andersen, H. (1964a).
The human fetal thyroid 1- Its weight in relation to body weight, crown-rump length, foot length and estimated gestation age.
Anatomical Record 148, 123-128.

Shepard, T.H., Andersen, H.T. and Andersen, H. (1964b).
Histochemical studies of the human fetal thyroid during the first half of fetal life.
Anatomical Record 149, 363-380.

Silva, E. (1972).
Disposal rates of T4 and T3 in iodine-deficient rats.
Endocrinology 91, 1430-1435.

Silva, J.E. and Matthews, P. (1984).
Thyroid hormone metabolism and the source of plasma T3 in 2-week old rats: effects of thyroid status.
Endocrinology 114, 2394-2405.

Singer, P.A. and Nicoloff, J.T. (1972).
Estimation of T3 secretion rate in euthyroid man.
Journal of Endocrinology and Metabolism 35, 82-89.

Slebodzinski, A.B. (1979).
Metabolic response to thyroxine in the newborn pig.
Biology of The Neonate 36, 198-205.

Slebodzinski, A., Nowak, G. and Zamystowska, H. (1981).
Sequential observation of changes in T4, T3 and rT3 during the postnatal adaptation of the pig.
Biology of The Neonate 39, 191-199.

Smith, R.E. and Horwitz, B.A. (1965).
Brown fat and thermogensis.
Physiological Review 49, 330-425.

Stevens, J.F. (1970).
Plasma cortisol levels in the neonatal period.
Archives of Diseases in Childhood 45, 592-594.

Strelin, K. and Larzaris, J.H. (1977).
The thyroid and its control.
Annual Reviews of Physiology 39, 349- 371.

Strott, C.A., Goff, A.K. and Lyons, C.D. (1981).
Functional differences between the outer and inner zones of the guinea pig adrenal cortex.
Endocrinology 109, 2249-2251.

Tanate, Y., Ishil, T. and Tomaki, Y. (1969).
Comparison of T4-binding plasma proteins of various vertebrates and their evolutionary aspects.
General and Comparative Endocrinology 13, 14-21.

Taurog, A. (1970).
Thyroid peroxidase and T4 biosynthesis.
Recent Progress in Hormone Research 26, 189-247.

Thomas, A.L., Comline, R.S., Silver, M. and Nathanielsz, P.W. (1974).
Plasma thyrtrophin concentration in the fetal calf.
Experientia 30, 1226-1228.

Thomas, A.L., Jack, P.M.B., Manns, J.G. and Nathanielsz, P.W. (1975).
Effect of synthetic thyrotrophin releasing hormone on thyrotrophin and prolactin concentrations in the peripheral plasma of the pregnant ewe, lamb fetus and neonatal lamb.
Biology of the Neonate 26, 109-116.

Thomas, A.L., Krane, E.J. and Nathanielsz, P.W. (1978).
Changes in the fetal thyroid axis after induction of premature parturition by low dose continuous intravascular cortisol infusion to the fetal sheep at 130 days of gestation.
Endocrinology 103, 17-23.

Thomas, A.L. and Nathanielsz, P.W. (1982).
"Thyroid function in the mammal - its role in normal development"
In:- Veterinary Medicine (eds Phillipson, A.T., Hall, L.W. and Pritchard, W.R.) Pub. Heinemann pp 7-13.

Thomas, A.L. and Nathanielsz, P.W. (1983).
"The Fetal Thyroid".
In:- Current Topics in Experimental Endocrinology.
(eds Martini, L. and James V.H.T.) Pub Academic Press 5, 97-116.

Thomas, S.J., Wilson, D.W., Pierrepoint, C.G., Cameron, E.H.D. and Griffiths, K.J. (1976).
Measurement of cortisol, cortisone, 11-deoxycortisol and corticosterone in fetal sheep during perinatal peroid.
Journal of Endocrinolgy 68, 181-189.

Thorburn, G.D. and Hopkins, P.S. (1973).
Thyroid function in the fetal lamb.
In:- "Fetal and Neonatal Physiology".
" Sir Joseph Barcroft Centenary Symposium" (eds R.S. Comline K.W. Cross, G.S. Dawes and P.W. Nathanielsz) pp 488-507.

Thorburn, G.D., Waters, M.J., Young, I.R., Dolling, M., Buntine, D. and Hopkins, P.S. (1981).
"Epidermal Growth Factor: A critical factor in fetal maturity."
In:- Ciba Foundation Symposium- The Fetus and Independent Life.
pp 173-202.

Tice, L.W. and Wollman, S.H. (1974).
Ultrastructural localization of peroxidase on pseudopods and other structures of the typical thyroid epithelial cell.
Endocrinology 94, 1555-1567.

Uhrmann, S., Marks, K.H., Maisels, M.J., Friedman, Z., Murray, F., Kulin, H.E., Kaplan, M. and Utiger, R. (1978).
Thyroid function in the preterm infant: a longitudinal assessment.
Journal of Pediatrics 92, 968-973.

Vagenakis, A.G., Burger, A., Portnay, G.T., Rudolph, M., O'Brian, J.T. Azizi, F., Arky, R.A., Nicod, P., Ingbar, S. and Braverman, L.E. (1975).
Diversion of peripheral T4 metabolism from activating to inactivating pathways during complete fasting.
Journal of Clinical Endocrinology and Metabolism 41, 191-194.

Vale, W., Burgus, R. and Guilleman, R. (1968).
On the mechanism of action of TRF: effects of cycloheximide and actinomycin on the release of TSH stimulated in vitro by T4.
Neuroendocrinology 3, 34-36.

Vale, W., Spiess, J., Rivier, C and Rivier, J. (1981).
Characterisation of a 41-residue ovine hypothalamic peptide that stimulates secretion of ACTH and Beta endorphin.
Science 213, 1394-1397.

Van Herle, A.J., Young, R.T., Fisher, D.A., Uller, R.P. and Brinkman, C.R. (1975).
Intra-uterine treatment of a hypothyroid fetus.
Journal of Clinical Endocrinology and Metabolism 40, 474-477.

Vanderlaan, J.E. and Vanderlaan, W.P. (1947).
The iodide concentrating mechanism of the rat thyroid and its inhibition by thiocynate.
Endocrinology 40, 403-416.

Van Middlesworth, L. (1957).
T4 excretion, a possible cause of goitre.
Endocrinology 61, 570-573.

Van Wagenen, G. and Newton, W.H. (1943).
Pregnancy in the monkey after removal of the fetus.
Surgery, Gynecology and Obstetrics 77, 539-543.

Van Wyk, J.J., Arnold, M.B., Wynn, J. and Petter, F. (1959).
Effects of soybean produce on thyroid function in humans.
Pediatrics 24, 752-760.

Walton, K.W., Scott, P., Dykes, P.W. and Davies, J.W.L. (1965).
The significance of alterations in serum lipids and thyroid dysfunction. Alteration in metabolism and turnover of ^{131}I low-density lipoproteins in hypothyroidism and thyrotoxicosis.
Clinical Science 29, 217-238.

Warren, J.C. and Cheatum, S.G. (1967).
Maternal urinary oestrogen secretion: effect of adrenal suppression.
Journal of Clinical Endocrinology and Metabolism 27, 433-436.

Waterman, A.J. and Gorbman, A. (1956).
Development of the thyroid gland of the rabbit.
Journal of Experimental Zoology 132, 509-538.

Webb, P.D. (1980).
Development of the adrenal cortex in the fetal sheep: an ultrastructural study.
Journal of Developmental Physiology 2, 161-181.

Webster, R.C. (1949).
Growth and time of sexual maturation in goitrous guinea pigs.
Anatomical Record 103, 517.

Webster, R.C. and Young, M.C. (1948).
Thiouracil treatment of the female guinea pig: effect on gestation and the offspring.
Anatomical Record 101, 722.

Weiss, R.M. and Noback, C.R. (1949).
The effect of T4 and thiouracil on the time of appearance of ossification centres of rat fetuses.
Endocrinology 45, 389-395.

Welch, R.A.S., Forst, O.F. and Bergman, M. (1973).
The effect of administering ACTH directly to the fetal calf.
New Zealand Medical Journal 78, 365. Abstract.

Wells, L.J. (1947).
Progress of studies designed to determine whether the fetal hypophysis produces hormone that influence development.
Anatomical Record 97, 409-421.

Westphal, U. (1967).
Steroid-protein interactions X111. Concentrations and binding affinities of corticosteroid-binding globulins in sera of man, monkey, rat, rabbit and guinea pig.
Archives of Biochemistry and Biophysics 118, 556-567.

Widdowson, E.M. (1968).
Growth and composition of the fetus and newborn.
In "Biology of Gestation" (ed N.S. Assali) pp 1-49.

Wiersinga, W.M., Modderman, P. and Touber, J.L. (1980).
The effect of α and β adrenoceptor agonists, and antagonists on the *in vivo* conversion of T4 to T3.
Hormone and Metabolic Research 12, 346-347.

Wigglesworth, J.S. (1966).
Fetal growth retardation.
British Medical Bulletin 22, 13-15.

Willcox, D.L., Yovich, J.L., McColm, S.C. and Schmitt, L.H. (1985).
Changes in total and free concentrations of steroid hormones in the plasma of women throughout pregnancy: effects of medroxy-progesterone acetate in the first trimester.
Journal of Endocrinology 107, 293-300.

Wolff, J., Chaikoff, I.L., Coldberg, R.C. and Meier, J.R. (1949).
The temporary nature of the inhibitory action of excess iodide on organic iodine synthesis in the normal thyroid.
Endocrinology 45, 504-513.

Wolff, J., Chaikoff, I.L., Taurog, A. and Rubin, L. (1946).
The disturbance in iodine metabolism produced by thiocyanate: the mechanism of its goitrogenic action with radioactive iodine as indicator.
Endocrinology 39, 140-148.

Woods, R.J., Sinha, A.K. and Ekins, R.P. (1984).
Uptake and metabolism of thyroid hormones by the rat fetus in early pregnancy.
Clinical Science 67, 359-363.

Wrutniak, C. and Cabello, G. (1983).
changes in the concentration of T4 in the plasma of rat fetuses during late gestation: influence of ligation of the maternal uterine vein and artery.
Journal of Endocrinology 99, 233-238.

Wu, B., Kikkawa, Y., Orzalesi, M.M., Motoyama, E.K., Kaibara, M., Zigas, C.J. and Cook, C.D. (1973).
Effect of T4 on maturation of fetal rabbit lung.
Biology of the Neonate 22, 161-168.

Wu, S.Y., Klein, A.H., Chopra, I.J. and Fisher, D.A. (1977).
In Proceedings of The 53rd meeting of The American Thyroid Association, September 7-10, (Abstract T-15).

Wu, S.Y., Polk, D.H., Klein, A.H. and Fisher, D.A. (1986).
The mechanism of low serum T3 in the fetus: hepatic T4 5-monodeiodinase versus tissue sulfhydryl content-a clarification.
Journal of Developmental Physiology 8, 43-47.

Yamada, T., Kajihara, A., Takemura, Y. and Onaya, T. (1975).
Antithyroid compounds.
In:- American Handbook of Physiology, Section 7,
Endocrinology III, pp 345-357.