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Developmental changes in the membrane fatty acids of rats and the role of the thyroid

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Developmental changes in the membrane fatty acids of rats and the role of the thyroid.

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DECLARATION

The work presented in this thesis, in fulfilment of conditions governing the award of the degree of Master of Science, has not been submitted at any other university or institution for a higher degree except where specifically indicated.

NAMITA SEN.

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Chapter 1

REVIEW OF LITERATURE

1.1 Membrane lipids.

1.1.1 Structure,Function And General Consideration Of Membrane Lipids.

The important role of the lipid component in the functioning of cell membranes has been recognized since the work of Overton in the 1890's (Overton 1899) who showed that the ability of compounds to permeate cell membranes was related directly to their lipid solubility. The structural basis for this role was first indicated by the work of Gorter and Grendel (1925) who showed that the amount of lipids extracted from red blood cell membranes was sufficient to form a continuous bilayer.

The first important hypothesis of the structure of a biological membrane which featured a bilayer of lipid coated at its aqueous interfaces with layers of protein, was proposed by Danielli and Davson (1935). Later on Stein and Danielli (1956) suggested that protein might penetrate into or through the lipid bilayer. This was not based on any direct knowledge of

membrane proteins but was simply a speculative attempt to account for the occurence of facilitated permeation of solutes through the plasma membrane.

Robertson in 1964 postulated the unit membrane structure in order to account for layered structure of cell membrane (Finean, 1962) under the electron microscope. According to this concept, which was proposed in the 1950s, membranes contain a bilayer of phospholipid molecules in which the hydrophobic hydrocarbon chains of the lipid face each other to form a continuous hydrocarbon barrier. Such a lipid layer is oily in nature and cannot conduct an electric current. He also proposed that the lipid bilayer is coated on each side with a single layer of extented protein molecules. Many models were proposed between 1964 and 1972 (as shown in figure 1) but these models failed to account for many properties of membranes (Lucy, 1964; Sjostrand,1965; Green and Perdue,1966; Lenard and Singer,1966; Benson,1968; Vanderkooi and Green,1970, and Finean,1972.). Singer and Nicolson (1972) proposed the fluid mosaic model which is now widely accepted. This model postulates that the phospholipids of membranes are arranged in a bilayer to form a fluid, liquid

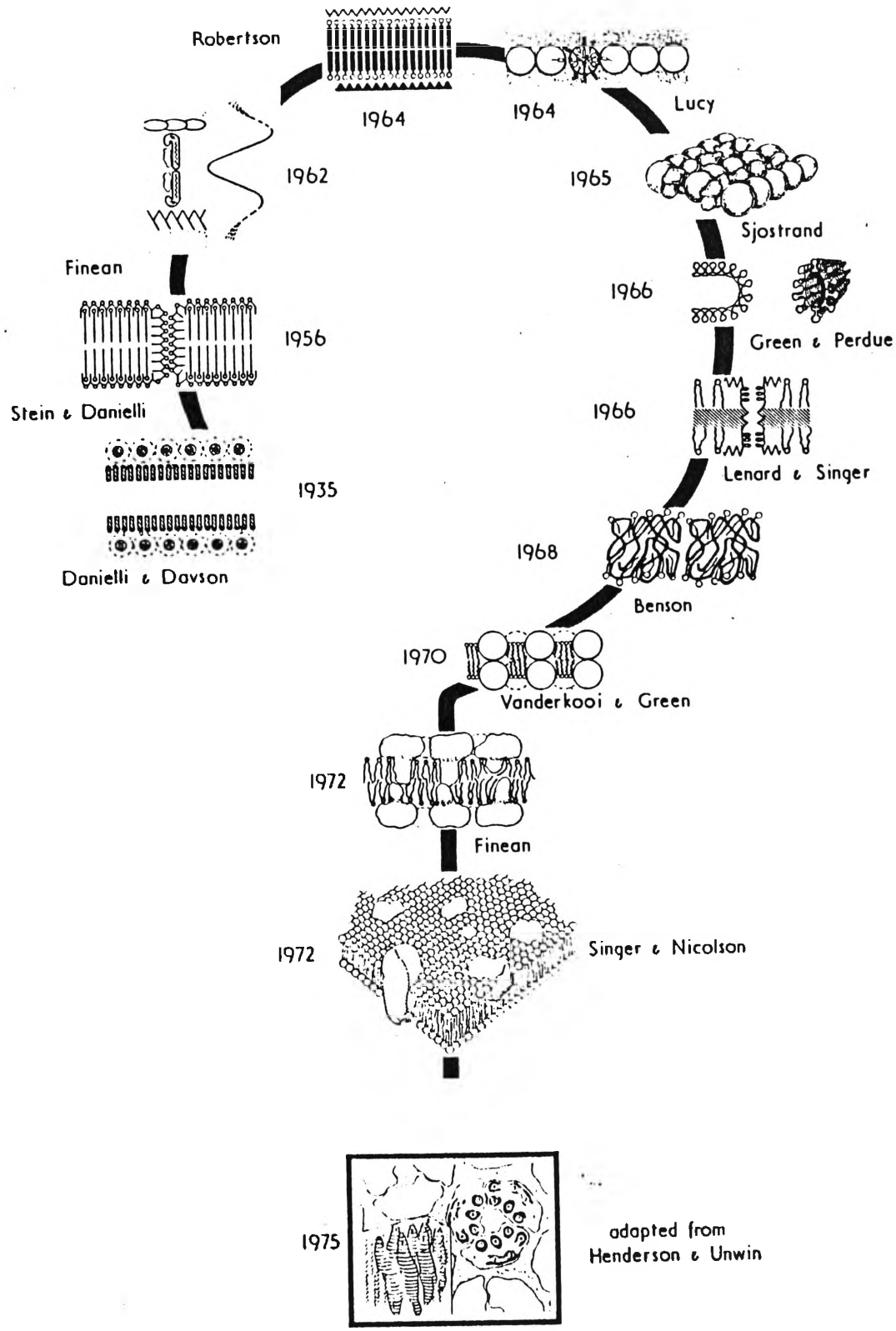

Figure 1 : Diagram illustrating the chronological order in which the most influencial models have been proposed (Membrane structure : Edited by Finean & Michell, 1981).

crystalline matrix or core. In this bilayer individual molecules can move laterally, endowing the bilayer with fluidity, flexibility and a characteristically high electrical resistance and relative impermeability to highly polar molecules. The principal feature of this model is that the lipids in membranes are present in the form of a bilayer, with the membrane proteins either bound to the charged surface of the bilayer (peripheral proteins) or inserted to varying degrees (integral proteins) into the bilayer. There is considerable experimental evidence for this model (Hendler, 1971, 1974; Singer, 1971,1974).

In 1975, Henderson and Unwin described the specific structure of the purple membrane of Halobacterium halobium. As experimental methods have improved it is now becoming possible to describe structural patterns of specific membranes which differ both in the spatial distributions of their molecular components and in the mobilities of these components.

Many physiological and biochemical processes of living organisms are influenced and/or regulated by cellular membranes (Platt-Aloia and Thomson, 1981). The separation of

a cell's contents from its surroundings is the most critical function of cell membranes (Esser Alfred, 1982). The cell membranes serve many functions including permeability, transport, excitability, intracellular interaction, morphological differentiation, fusion (Jain 1979) (see figure 2) and receptor sites for macromolecular signals (Maclennan et al. 1971; Spatz and Strittmatter, 1973; Hynes, 1976; Oppenheim and Rosentreich, 1976; Bergman and Haimovich, 1977; Ishii and Nakae, 1980).

Phospholipids are generally only found in membranes (Dawson and Lindsay, 1960) and generally contain one saturated and one unsaturated acyl chain. In animal cell membranes, the acyl chain at the Sn-2 position is unsaturated (Lands and Hart, 1966; Tattrie et al.1968). There is no preferential pairing of any particular saturated chain with any particular unsaturated chain. Molecules of reverse distribution or with two identical fatty acids are found in phospholipids (Montfoort et al. 1971; Van deenen 1971; Holub and Kuksis, 1978).

Lipids account for about half of the mass of most membranes (Lehninger, 1975). In internal membranes the lipid is almost exclusively phospholipids whilst cytoplasmic

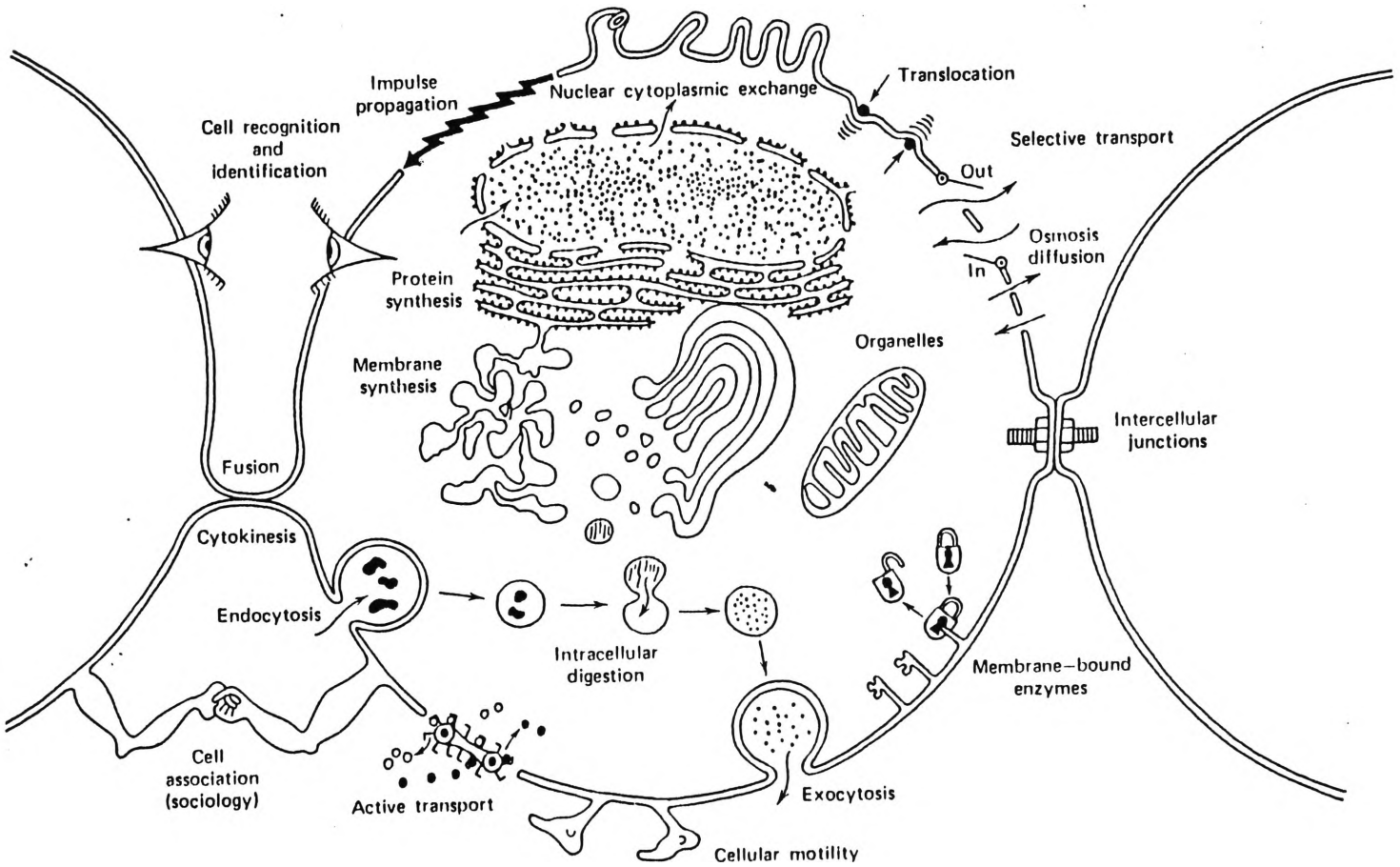

Figure 2 : Various processes modulated by cell membranes (Introduction to biological membranes : Edited by Jain and Wagner, 1980).

membranes, in contrast, contain both a small amount of glycolipid and neutral lipids including cholesterol in addition to phospholipids (Bretscher, 1971). Generally all mammalian tissues and organelles contain qualitatively the same lipids, except for diphosphatidyl-glycerol which predominates in mitochondrial inner membranes, lysosomal membranes and the microbial membranes. Phosphatidylcholine is the major phospholipid of animal membranes, phosphatidylethanolamine is the major phospholipid of bacterial membranes and glycolipids predominate in plant membrane. Cholesterol occurs in animal plasma membranes, lysosomal membranes and golgi membranes (Korn, 1969). Sphingomyelin and gangliosides are usually found in plasma membranes of animal cells but seldom in plant or bacterial membranes (Chapman, 1982).

In most naturally occuring lipids, the acyl chains are even numbered. Although odd numbered fatty acids do occur naturally, their proportion in membrane phospholipids rarely exceeds 2%. The C-16, C-18 and C-20 fatty acids constitute more than 80% of the acyl chains in most membranes (Chapman, 1982). Unsaturated acyl chains with one to six double bonds are also present in membranes. However, 18 : 1, 18 : 2, 18 : 3

and 20 : 4 species constitute more than 90% of the unsaturated acyl chains in most membranes (Chapman, 1982).

The presence of branched chain and cyclic fatty acids in membrane phospholipids is rare (Chapman, 1982). Cyclopropane rings containing fatty acids are probably present in bacteria such as E.coli. In some bacteria a number of unusual fatty acids containing methyl branches or extremely long chains have also been found (Davis et al. 1968). The proportion of saturated to unsaturated acyl chains changes considerably from one membrane to the other. In mammals, myelin and plasma membranes have a higher proportion of saturated acyl chains whereas inner mitochondrial membrane contain a higher proportion of unsaturated acyl chains (Chapman, 1982).

Membrane lipid composition varies from organ to organ in the same species, as well as in different species. This is true even when the classes of lipids are the same in the different membranes. Individuality is thus expressed most clearly in difference in fatty acid composition (Rothfield, 1971). Quite extensive changes in the lipid class and acyl chain compositions can be brought about by different dietary, environmental (temperature, pH and ionic strength), genetic,

hormonal, drug, age, physiological and pathological states (Jain, 1979). Most cells contain enzymes which can remove or exchange either one or both acyl chains and modify or exchange the base in phospholipids (Jain, 1979).

Many membrane bound enzymes have been found to require lipids to function properly (Coleman, 1973). A number of enzymes involved in oxidative phosphorylation contain phospholipids (Marinelti et al. 1958; Fleischer et al. 1961). Mitochondria and enzymes involved in electron transport are inactivated if phospholipids are removed. However, activity is restored with the addition of phospholipids (Fleischer et al. 1962; Reich and Wainio, 1961).

1.1.2 Phase Transition Temperature and Membrane Fluidity.

The state of the lipid in a membrane (whether it is crystalline or liquid crystalline) has a marked effect on the function of the membrane. Small molecules can easily pass through the membrane if it is in the liquid crystalline state but not if lipids are in the gel state (Lee, 1975). The way in which the transition temperature (the temperature at which changes

from the gel state to the liquid crystalline state occur) shifts up or down depending upon chain length, unsaturation and polar groups of the membrane lipids has been well studied (Oldfield and Chapman, 1972).

Highly unsaturated lipids have a low transition temperature and saturated lipids have a high transition temperature. Transition temperature increases with increasing fatty acid chain length. Transition temperature also depends on the chemical nature of the lipid polar head group (Ladbrooke and Chapman, 1969; Hinz and Sturtevant, 1972). X-ray crystallographic studies have revealed that below the phase transition fatty acyl chains in phospholipids are all in the trans conformation and are tightly and regularly packed in a crystalline array (Tardieu, 1973; Hauser et al. 1981).

At the transition temperature, there is a 50% increase in the surface area occupied by the lipid (Levine, 1972) and appreciable motion becomes possible about the C-C bonds of the fatty acid chains. Early studies from 1964 to 1968 with techniques such as infrared spectroscopy and nuclear magnetic resonance indicated a number of important features of phospholipid molecules when these molecules

exceed a certain critical transition temperature (Chapman et. al. 1967; Chapman and Penkett, 1966., Chapman and Salsbury,1966).

Above the phase transition temperature : a) the lipid chains show flexing and twisting of the methylene CH_2 groups and a marked increase of rotational isomers; b) the oscillations and rotational disorder of the methylene groups are most marked at the methyl end of the lipid chains (Chapman and Penkett,1966., Chapman and Salsbury,1966). ; c) In addition to the chain motion, other parts of the molecule e. g. the polar group of lecithin molecules exhibit a marked increase in mobility (Veksli et al. 1969); d) Lipid self diffusion occurs when sufficient water is present to weaken any ionic linkages between the polar groups (Penkett et al. 1968). With this type of physical information, a fluidity concept was proposed which suggested that the particular distribution of fatty acyl residues that occurs with a particular environmental temperature, matches the required diffusion rate or rate of metabolic processes required by the tissues (Chapman and Penkett,1966., Chapman and Salsbury,1966).

Recent studies have defined some of the factors that determine the biophysical properties of cell membranes (Shinitzky, 1979; Shinitzky and Henkart, 1979). These include : a) the degree of unsaturation and length of the phospholipid acyl chains (Cogan et al.1973; Lentz et al.1976); b) the ratio of cholesterol to phospholipid (Vanderkooi et al.1974; Shinitzky and Inbar 1976); c) the ratio of lecithin to sphingomyelin (Shinitzky and Barenholz, 1974; Schmidt et al. 1977); and d) the ratio of lipid to protein (Shinitzky and Inbar,1976).

Membrane fluidity depends on the degree of unsaturation of the phospholipid acyl chains (Chapman and Wallach, 1968). Double bonds of natural fatty acids are virtually all of the cis-configuration and their presence in phospholipids increases fluidity and decreases the degree of order (restriction of mobility) in the system. Thus replacement of stearic acid (18 : 0) with oleic acid (18 : 1) markedly increases the fluidity, but further replacement of oleic acid with linoleic acid (18 : 2) has only a small effect (Stubbs and Smith, 1984). This example serves to illustrate how minor modification of membrane components can change the detailed properties of a membrane without changing the overall structure (Housley and Stanley, 1982).

The position of double bonds may be a more important influence in some cases than the actual number of double bonds, the nearer the double bond to the centre of the chain, the lower the melting point (Christie and Holman, 1967). Thus the melting point (-10°C) of 18 : 3 Δ 9, 12, 15 is not markedly different from that of 18 : 2 Δ 9, 12 but is some 28° C lower than that of 18 : 2 Δ 12, 15. A recent study (Coolbear et al. 1983) has shown that the introduction of the first double bond into distearoyl phosphatidylcholine lowers the phase transition temperature by nearly 50°C and two double bonds (18:0\18:2 phosphatidylcholine) lowers it by a further 22°C, three or four bonds bring about no further decrease and in fact cause a slight increase.

The degree of unsaturation of phospholipid acyl chains can be efficiently modulated by intracellular metabolism which is now believed to be the main regulatory mechanism of membrane fluidity in adaptation to temperature (Hazel and Posser, 1974; Sinensky, 1974; Cossins, 1977) and metabolic or nutritional disorders (Cooper et al. 1977).

It is important to appreciate that the fluidity of lipids of cell membranes is not, however, solely related to acyl

chain length or unsaturation . In membranes such as myelin where the lipids are more saturated, the lipid would be rigid at body temperature except for the presence of cholesterol (Chapman, 1975). In this membrane the cholesterol keeps the lipids in a fluid condition by interposing between the lipid chains to prevent chain crystallization from occuring (Ladbrooke et al. 1968 b).

In fact cholesterol appears to have a dual effect on lipid systems in that at a temperature where the lipid would normally be in a gel condition, the presence of cholesterol causes the lipid to be in a fluid condition (Ladbrooke et al. 1968,a) whereas at a temperature where the lipid is in the fluid condition the cholesterol inhibits some of the chain molecular motion although fluid characteristics are still retained (Chapman and Penkett, 1966; Williams and Chapman, 1970; Chapman, 1973). In most mammalian systems under physiological conditions, cholesterol acts as a fluidizer by disturbing the structural regularity of the membrane (Albert et al.1983).

The ratio of lecithin to sphingomyelin also affects membrane fluidity. These two phospholipids constitute more

than 50% of the phospholipids in mammalian membranes (Rouser et al. 1968) and about 90% of mammalian serum phospholipids (Nelson, 1967). Their fluidity properties are different because of inherent structural differences.

Natural lecithin has an unsaturated fatty acid specially at the SN-2 position which causes increasing fluidity, whereas natural sphingomyelin bears saturated fatty acids which cause low fluidity in the lipid domain and displays a broad phase transition between 25°C and 35°C (Shinitzky and Barenholz, 1974; Schmidt et al. 1977). Furthermore at 37°C, where sphingomyelin is mostly in a fluid phase, it still possesses a fluidity which is about 6-fold lower than natural lecithin (Shinitzky and Barenholz, 1974).

The rigidifying effect of sphingomyelin is only partially due to its highly saturated hydrocarbon chains. Inter and intramolecular hydrogen bonding between its amide linkage and the free hydroxyl group, in addition to the sphingosine trans double bond, condense the hydrocarbon-water interface region which presumably confers rigidity on the hydrocarbon region as well (Shinitzky and Barenholz, 1974).

The dynamic characteristics of the membrane lipid bilayer are indirectly affected by the presence of proteins. Protein decreases fluidity and decreases the flow activation energy (Borochov and Shinitzky, 1976). When the membrane fluidity increases, the bulk proteins will sink into the membrane interior (Shinitzky,1979; Yasuda et al. 1977; Brulet and Mcconnell, 1977). This has physiological significance in adaptation of membrane receptors, antigens and enzymes and extention to functional lipids (Shinitzky, 1979; Yasuda et al. 1977; Brulet and mcconnell, 1977).

Damel and Dekruijff (1976) have modified artificial membranes by adding cholesterol. They suggest that insertion of cholesterol into phospholipid membranes causes an intermediate gel state that is not affected by temperature. The second way to modify the membrane fluidity is by the addition of lysophosphatides (Weltzien,1979). Keith et al. (1975) found that the presence of 7% lysophosphatides in liver mitochondrial membranes of hibernating ground squirrels caused the loss of ability to exhibit a phase transition.

Molecules with quasispherical geometry possessing a polar group and a bulky nonpolar moiety have been shown by

both biological and physical methods of detection to be capable of lowering membrane phase transition temperatures (Eletr and Keith, 1972; Eletr et al. 1974). It has been found that many types of cell modify the composition of their membrane phospholipid fatty acids during short-term culture if the type of lipid in the culture medium is restricted and enriched with a particular fatty acid (Baily and Dunbar1973; Spector et al. 1981). Fatty acid composition in various membranes can also be altered by dietary manipulation (Brivio-Haugland et al. 1976; Withers 1977; Nouvelot et al. 1983; Tinoco 1983; Bourre et al. 1984).

1.2 Thyroid.

1.2.1 The Thyroid Gland- Hormone Biosynthesis and Secretion.

The fully developed thyroid gland is one of the largest of the body's endocrine organs. It is located anterior to the upper part of the trachea, near its junction with the larynx (Turner, 1976). The thyroid gland is divided into right and left lobes that are joined across the trachea by a thin band called the isthmus (Werner and Ingbar, 1978). The vascular supply to the thyroid is exceptionally rich. It is probable that more blood flows through this gland , in proportion to its size , than through any other organ of the body, with the possible exception of the adrenal gland. The most outstanding feature of the thyroid is its ability to concentrate large amounts of iodine, the amount of iodine within the gland may be fifty to several hundred times that in the blood plasma (Turner, 1976).

The basic internal structure of the thyroid consists of hollow balls of cells called follicles that are bound together with connective tissue. The thyroid gland is different from all other endocrine glands in having extracellular storage sites

(the central region of the follicles) for its hormones. The other endocrine glands store their hormones within the cells of the gland (Turner, 1976). The central region of each thyroid follicle contains a protein substance, the colloid, which is a stored form of thyroid hormones. The gland is small and difficult to separate accurately from the adjacent muscles, especially in the younger stages. In human, the thyroid is relatively large in the newborn, decreasing from about 0.04% of body weight to 0.018% at one year (Jackson, 1913). The thyroid apparently increases only slightly during first week while the body weight doubles. No difference according to sex is apparent (Jackson, 1913). It has also been shown that thyroid of adult animals decrease in size (weight) between maturity and senescence (Bourne 1967; Haensley and Getty, 1970; Irvine, 1973; Pittman, 1962).

Biosynthesis of thyroid hormones takes place by the following steps : a) active transport of iodide into the thyroid; b) oxidation of iodide and iodination by the oxidized form of tyrosyl residues within thyroglobulin to yield the hormonally inactive iodotyrosines and c) coupling of iodotyrosines to form

hormonally active iodothyronines, notably T_4 and T_3 (Pitt River, 1961; Tong et al. 1962).

Release of hormones, in addition, involves two additional groups of reactions; a) hydrolysis of thyroglobulin by a thyroid protease and by peptidases, liberating free iodinated amino acids; b) passage of iodothyronines into the blood, while the iodotyrosines undergo intrathyroidal deiodination (Taurog, 1978) (see figure 3)

The thyroid gland of fetal and newborn mammals are often highly hyperactive compared to the adult. For example fetal turnover of T_4 in the sheep exceeds maternal turnover by eight times (Dussault et al. 1972). However, in the rat thyroid secretion rate per unit body weight is quite low at birth and does not begin to increase until about 10 days of age, by which time birth weight has doubled (Beltz and Reineke, 1968). Other observations indicate that in rat, secretion rate is unchanged or declines slightly from about two to three months of age to midlife at about one year (Gregerman, 1963; Johnson et al. 1966). In adult animals of some species, thyroid activity appears to decrease with increasing age (Flamboe and Reineke,1959; Henneman et al. 1955; Pipes et al. 1963; Turner, 1948; Wilansky et al. 1957).

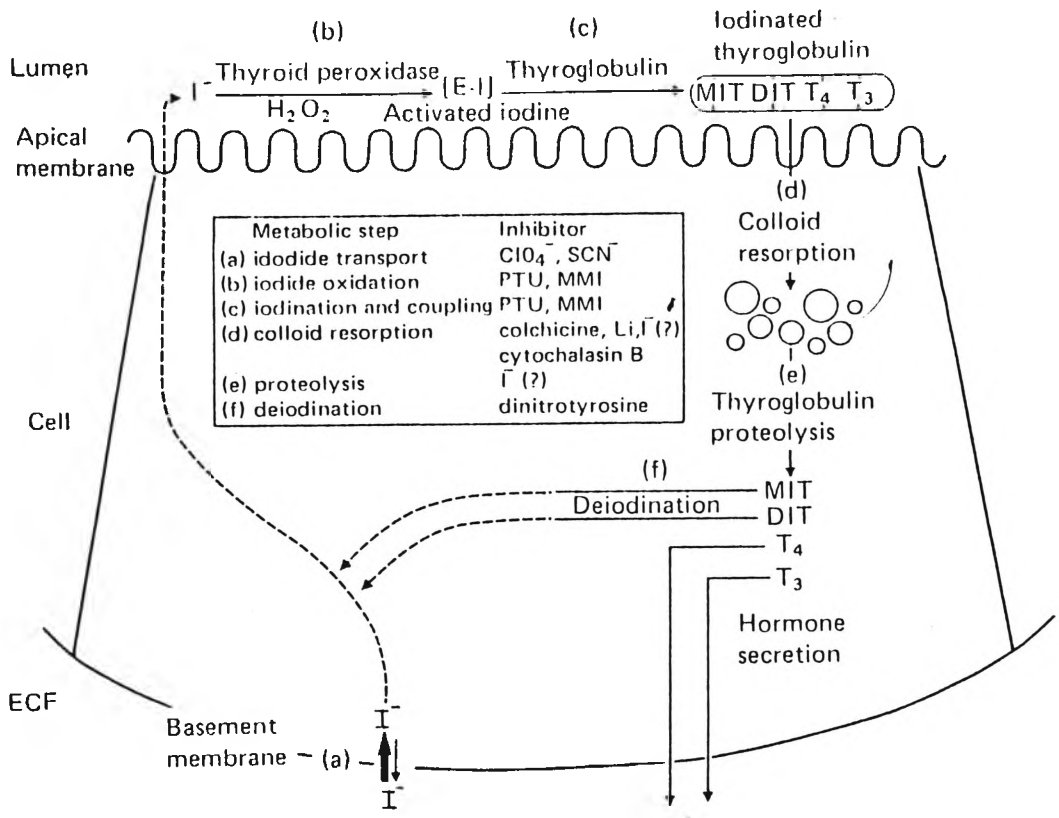

Figure 3 : Diagramatic representation of steps in thyroid hormone biosynthesis and release (Modified from Tong, W : Thyroid hormone synthesis and release. In Werner SC, Ingbar SH (eds) The Thyroid, 3rd. ed. New York, Harper & Row, 1971, p.24).

1.2.2 Effect Of Thyroid Hormones On Growth and Development.

The secretions of the thyroid gland, L-thyroxine (T_4) and L-triiodothyronine (T_3) are known to influence the structure, function and development of most organs and tissues of vertebrate species (Oppenheimer and Surks, 1975). The nature of these effects is quite variable (Oppenheimer and Surks, 1975). Thyroxine was, for a long time, thought to be a true growth hormone. This view was based on the short stature of cretins and the delayed growth of thyroidectomised animals (Turakulov et al. 1975). Thyroid hormones are responsible for initiation of amphibian metamorphosis (Etkin, 1968; Frieden and Just, 1970; Tata, 1971; Cohen, 1970). In the mammalian fetus they are essential for the late structural development and the organization of the neuronal elements of the central nervous system (Eayrs, 1960; Hamburgh, 1969) and the appearance of primary centres of ossification in bone (Wilkins, 1941).

In postnatal development, thyroid hormones are required to establish normal rates of linear growth (Wilkins,

1955). Rats thyroidectomised soon after birth lag behind their normal counterparts in rate of gain of body weight and body length and the ultimate size attained is significantly diminished (Scow et al. 1949; Ray et al. 1950; Scow, 1951; Scow and Simpson, 1945). Administration of growth hormone to neonatally thyroidectomised rats stimulates a significant gain in growth as measured by increased body weight and length (Scow and Marx, 1945; Scow,1951,1959; Ray et al. 1950,1954). Thyroid hormone administration causes only a minimal stimulation of growth in absence of growth hormone (Scow, 1959; Thorngren and Hanssen, 1973). However, there is evidence to suggest that growth hormone is significantly more effective in the presence of thyroid hormone than in its absence (Scow, 1959; Evans et. al. 1939; Scow et al. 1949; Ray et al.1954; Thorngren and Hanssen, 1973).

Animals given both hormones grow at a much greater rate than those given maximal doses of either one alone. Martin and Wilkins (1958) and Van den Brande et al. (1973) demonstrated that thyroid hormone administration has little effect on the growth rate of growth hormone deficient children. On the other hand, the presence of thyroid hormone was required for an optimal response to growth hormone.

The mechanism by which thyroid hormones increase the responsivity of the animal to growth hormone is unknown. Recently it has been shown that thyroid hormone can modulate the concentration of prolactin and other plasma membrane receptors (Duran-Garcia et al. 1979; Sharma and Banerjee, 1978; Marshall et al. 1979; Williams et al. 1977). Although plasma membrane receptors for growth hormone have been identified in lymphocytes (Mc. Guffin et al. 1976), adipocytes (Fagin et al. 1980) and hepatocytes (Ranke et al. 1976; Postel Vinay and Desbuquois,1977), there is no evidence to date of modulation of receptor concentration in these tissues by thyroid hormone. In any case biochemical studies of liver indicate that in this tissue, growth hormone and thyroid hormones act in an additive fashion to stimulate synthesis of total and poly A^+ containing RNA (Simat et al. 1980) as well as the activity of RNA polymerase (Windell and Tata, 1966).

In the adult, both decreased and excessive thyroid hormone secretion have widespread physiological and anatomical effects in almost all tissues. However, the effects of loss of thyroid function or deficiency of thyroid hormones in the body differ depending on the animal's age. The younger the

animal, the more severe is the effect of thyroid deficiency on its general condition (Turakulov et al.1975).

Wysocki and Segal (1972) have suggested that the concentration of thyroid hormones increase dramatically during development prior to weaning initiation. Thyroid hormones have also been shown to induce maturational changes in various organ systems during the third postnatal week (Henning, 1981). Moreover, it has been observed that thyroid hormones affect the timing of weaning (Allen et al. 1978; Hemon, 1976; Wakelam et al. 1979) however, these reports involved only passing observations and no data were provided. It has been proposed by Blake and Henning in 1983 that timing of weaning is independant of thyroid hormones. But a recent study shows that the weaning time period is shorter for hypothyroid pups (7 days) than it is for control pups (9 to10 days). (Blake and Henning 1985). They also show that weaning initiates on day 17 and completes on day 26. Thus hypothyroidism delays but does not abolish the weaning process (Blake and Henning, 1985). However, weaning initiation is delayed 5 days in hypothyroid pups as compared with euthyroid pups.

Blake and Henning (1985) also show that 0.001% PTU in drinking water serves as an excellent means of inducing

hypothyroidism without the weakness characteristic of pups raised on 0.01% PTU or higher in the drinking water which has been used on developmental studies in the infant rat to induce hypothyroidism (Blake and Henning, 1983; Coulombe et al.1980; Henning, 1978; Romano and Henning, 1984; Swenne, 1983; Wysocki, 1972; Moussavi et al.1985). PTU (0.001%) in drinking water lowers serum T_4 concentration 25% as compared to euthyroid rats. Such hypothyroid pups continue to grow but are significantly smaller than the controls (Blake and Henning, 1985).

1.2.3 Use Of An Antithyroid Agents For A Chemical Thyroidectomy.

A wide variety of chemical agents have the ability to inhibit one or more reactions required in the synthesis of thyroid hormones (Leathem, 1958) but do not have major adverse effects on other organ systems (Mackenzie and Mackenzie, 1943). Some caution must be exercised, however, in that some goitrogens have been shown to produce effects on other tissues (especially, liver) that do not appear following

surgical thyroidectomy (Hopper and Yatvin, 1965; Yatvin et al. 1964). The most potent are thioureylenes defined by the grouping S=C<, several of which are used in the treatment of hyperthyroidism (Stanley and Astwood, 1947).

The mode of action of these agents on hormone biosynthesis is complex (Taurog, 1970; Taurog, 1974). Although initially considered to exert their antithyroid action solely by inhibiting the oxidation (Alexander, 1959; Coval and Taurog, 1967; Degroot and Davis, 1962; Hosoya, 1963; Morris et al. 1966) and organic binding of thyroid iodide, they are now known to inhibit the coupling of iodotyrosines primarily and the formation of DIT and MIT secondarily (Richards and Ingbar, 1959). Thus they are capable of producing an inhibition of hormone synthesis to far greater degree than the inhibition of total iodine accumulation.

In addition to inhibiting hormone synthesis, propylthiouracil impairs the conversion of T_4 to T_3 in the peripheral tissue (Oppenheimer et al. 1972). This conversion is quite important because most of the action of thyroxine has been suggested to come from its conversion to T_3

(Oppenheimer et al. 1979; Silva and Larsen, 1978). Although thyroxine can bind to nuclear receptors and thereby exert some intrinsic effects, thyroxine has a lower affinity than T_3 (Oppenheimer et al. 1976).

The use of antithyroid drug (PTU) as a means of chemical thyroidectomy has an advantage over surgical thyroidectomy as it has been found that thyroidectomised animals can make thyroid hormones extrathyroidally (Taurog and Evans, 1967).

A large number of structural or functional abnormalities may lead to deficient production of thyroid hormones. The clinical state resulting therefrom is termed hypothyroidism. Causes of hypothyroidism can be divided into three categories; a) resulting from loss or atrophy of thyroid tissue; b) due to insufficient stimulation of an intrinsically normal gland as a result of hypothalmic or pituitary disease and c) associated with compensatory goitrogenesis as a result of defective hormone biosynthesis (Ingbar and Woebar, 1981).

The antithyroid drugs are used mainly for chemotherapy of thyrotoxicosis and as a substitute for

thyroidectomy in experimental animals particularly in newborn (Ingbar and Woebar, 1981). An aspect of great importance in relation to the use of these drugs is that they cross the placenta, are excreted in breast milk and are capable, therefore, of inhibiting thyroid function in the fetus and neonate (Marchant et al. 1977).

Weiss and Noback (1949) have shown that if the pregnant rat is treated with thiouracil, ossification in the 16 days old rat fetus is delayed, but Hamburgh et al. (1964) have shown that if there is such a delay, the hypothyroid fetus of the thiouracil treated mother has caught up with the untreated control at the time of birth. The hypothyroidism in the infant are usually self limited, disappearing soon after birth or after the child is weaned. Occasionally, however, extensive neonatal hypothyroidism may cause respiratory distress and death from asphyxia (Ingbar and Woebar, 1981).

It has been found that animals whose thyroid hormone production is impaired, 44% died during the 4th and 5th weeks. High mortality between ages 25 and 30 days has been found (Hughes, 1944). Animals that showed extreme retardation and had the drug withdrawn showed no recovery except one out of

seven. When thiouracil is withdrawn from older rats they finally assumed normal size and appearance.

1.3 Effect Of Thyroid Hormones On Fatty Acid Composition Of Membranes.

Many hormones affect cell surfaces so that the permeability of cell membranes, as well as the properties of the organelle surface within the cell, are altered (Ebling and Highman, 1971). It has been found that organelle membrane synthesis depends on adequate thyroid hormone levels ~~through~~ to enable the synthesis of phospholipids as well as of proteins. (Nelson and cornatzer, 1965; Tata, 1967). It has been shown some membrane systems are affected by thyroid hormones (Gruenstein and Wynn, 1970; Ismail Beigi and Edelman, 1970; Primack and Buchaman, 1974; Suko, 1971).

The way in which thyroid hormones exert their effect is poorly understood but it has been suggested they may operate by altering the fatty acid composition of cell membranes, thus changing membrane fluidity and function (Hulbert, 1978). Thyroid status results in an altered fatty acid composition of membrane phosphoglycerides (Peifer, 1968; Platner et al. 1972; Chen and Hoch 1977; Fass and Carter, 1981).

It has been found that in the rat the absence of thyroid hormones results an increase in the percent unsaturation of membrane fatty acids (Patton and Platner, 1970; Hulbert et al. 1976; Steffen and Platner, 1976; Chen and Hoch, 1977) whereas there is a decrease in unsaturation index of membrane fatty acids in hypothyroid rats as compared to control rats (Hoch et al. 1976; Chen and Hoch, 1976; Shaw and Hoch, 1976; Chen and Hoch, 1977; Hoch et al. 1980., Hoch et al. 1981; Hoch, 1982). Although these may seem to be contradictory results in that percent unsaturation and the unsaturation index would be expected to change in the same direction, sometimes an increase in the percent unsaturation of membrane fatty acids can occur with a decrease in the unsaturation index of the same membrane fatty acids (eg Table 1).

Since membrane fluidity is related to membrane fatty acid composition and since changes in membrane fatty acids induced by change in thyroid status are complex, it is obvious that changes in thyroid status will also be complexly related to changes in membrane fluidity.

Table 1 : Degree of membrane fatty acid unsaturation of various tissues.

Source	Membrane		% unsaturation		Unsaturation Index	
			C	H	C	H
Patton & Platner (1970)	Liver-mitochondria	Warm	66	68	-	-
		Cold	64	66	-	-
	Whole liver	Warm	67	68	-	-
		Cold	66	72	-	-
Steffen & Platner (1976)	Heart microsome	Warm	63	63	-	-
		Cold	61	60	-	-
	Heart mitochondria	Warm	67	67 -	-	-
Hulbert, Augee & Raison. (1976)	Liver mitochondria		61	65	-	-

Chen & Hoch (1976)	Liver inner membrane vesicles		51	47	203	181
Shaw & Hoch (1976)	Liver nuclei		52	58	169	157
Chen & Hoch (1977)	Liver inner mitochondria		62	67	208	181
Hoch Subramanian, Dopeshwarker & Mead (1981)	Liver microsomes		64	61	191	170
	Liver mitochondria	PL(TOTAL)	66	67	201	179
		PC	58	60	162	151
		PE	52	57	190	176
		CL	77	76	175	164
		PS	41	43	150	180
Hoch (1982)	Heart mitochondria		71	69	209	190

Hoch (1982) suggested that the decreased content of membrane phospholipid arachidonyl acyl chain (20 : 4) and increased linolenate (18 : 2) acyl group of mitochondria, microsomal and nuclear membranes prepared from livers or heart of hypothyroid rats confer a decrease in membrane fluidity because 20 : 4 acyl normally contributes more than half the unsaturation index.

Altered fatty acid composition of cell membranes can be accounted for at least in part by altered fatty acid desaturase enzymes (Fass and Carter, 1981) or altered secretion of very low density lipoproteins (Keyes and Heimberg, 1979; Schroeder et al. 1981). Another recent study has also suggested that thyroid hormone may regulate membrane fluidity by altering polyunsaturated fatty acid composition (Suzuki, 1982).

1.4 Changes In Membrane Fatty Acids During Development.

During early development in the mammals, dramatic alterations occur in the ratio of various phospholipid species,

in the degree of saturation of their acyl chains and in the cholesterol content of various biological membranes (Yarbro and Anderson, 1956; Novak et al. 1962; Dobiasova et al. 1964; Marshall, et al. 1966; Davison et al. 1966; Banik and Davison, 1967; Horrocks et al. 1966; Folch, 1955; Rovinski and Hosein, 1983; Kutchai et al. 1978; Garcia-Gonzalez et al. 1984).

It has been found that each organelle has its particular fatty acid composition (Tattin et al. 1981). This is in contrast with results which showed that fatty acid composition of phospholipid is the same in different organelles of the same tissue (Bartley, 1964). The composition may be profoundly influenced by the diet, but to different degrees in different organelles (Tattin et al. 1981). Changes in the diet at weaning have been suggested to cause some of the changes in fatty acid composition of membranes during this stage in postnatal development (Henning, 1981).

The aging process is also associated with the changes in the lipid composition of biological membranes. Changes in lipid composition occur both qualitatively and quantitatively with age, taking the form of altered cholesterol : phospholipid ratios, phosphatide ratios and degree of saturation of

phospholipid associated acyl chains. In general upon aging there is a tendency for a relative decrease in the more highly polyunsaturated fatty acids, while monounsaturated fatty acids and cholesterol : phospholipid ratio both increase (Doviasova et al. 1964; Garcia-Gonzalez et al 1984; Kutchai et al 1978). Bourre et al. (1984) has suggested that the saturated : monounsaturated fatty acid ratio which decreases with age might be an index of development. Membrane of aged animals therefore seem to be more rigid than their younger counterparts. Surprisingly, little attempt has been made to correlate alterations in the activity of membrane bound enzymes with the changes in the lipid environment of the membrane that occur during weaning and development.

Chapter 2

INTRODUCTION

As pointed out in chapter 1, many studies have shown that membrane fatty acid composition changes during development in mammals. As well, thyroid function also changes during development in mammals. Thyroid status is known to influence the fatty acid composition of a variety of membranes in adult mammals but, to my knowledge, has not been shown to influence membrane fatty acids in young developing mammals. The purpose of this thesis research was to determine if thyroid status influenced membrane fatty acid composition of the tissues of young developing rats and if the normal developmental changes in the tissue membrane fatty acids in the rat are due to changes in the mammal's thyroid activity.

The amount of work involved in such a study is large. Three tissues (liver, heart and brain) were chosen to be examined and changes in these tissues may be indicative of possible changes in other tissues. Rather than to isolate individual cellular membranes, the phospholipids from each

tissue were extracted and assumed to represent a composite membrane lipid fraction. The fatty acid composition of these phospholipids was determined to ascertain the nature of the membrane fatty acids of these tissues.

In any developmental study, the age classes chosen to be examined are critical. In the present study we were particularly interested in the influence of two events during the postnatal development of the rat. The first is the period of the development of homeothermy which is assosiated with a peak in plasma thyroxine level and occurs at approximately 10 days of age. The other event is the weaning process with its concomitant change in diet. In rats, prior to postnatal day 15, only milk is found in the stomach. The weaning process commences at about day 15 and is usually completed by day 25. (Blake and Henning, 1983; Blake and Henning, 1985). For these reasons, the rats were examined at the following ages; 1,5, 15, 25 days of age, as well as of adult rats. Thus it is hoped, the influence of these developmental events can be separated.

In order to determine the role of the thyroid in such changes, two groups were compared at each age. A normal euthyroid group were compared with a hypothyroid group, in

which thyroid function was inhibited by use of the antithyroid drug, propylthiouracil. This has been a common method of assessing the role of the thyroid in developmental studies (Agostino and Henning, 1982; Wysocki and Segal, 1972; Blake and Henning, 1983).

CHAPTER 3

METHODS

3.1 Materials

Rat diets were obtained from Allied feed (Rat and mouse cubes) Sydney, Australia. Ammonium molybdate powder, hexane, nitric acid, perchloric acid (70%), petroleum spirit, potassium dihydrogen orthophosphate, sodium chloride were purchased from Ajax chemical company. Acetic acid, acetone, ammonia, methanol were obtained from May & Baker. Ascorbic acid, butylated hydroxy toluene, propylthiouracil, Sil-LC silicic acid were obtained from Sigma. Boron trifluoride was purchased from BDH. Chloroform and ethyl ether anhydrous were purchased from Mallinckrodt. Fatty acid methyl esters were from Sigma chemicals and Amerlex T_4 and T_3 RIA kits were obtained from Amersham.

3.2 Animals

Rats were divided into two groups : a) control (euthyroid) pregnant animals were maintained on standard laboratory diet and drinking water ad libitum; b) experimental

(hypothyroid) animals were maintained the same diet as controls but their drinking water contained 0.05% (w/v) propylthiouracil from the fifteenth day of gestation. This procedure has been shown to cause hypothyroidism in both the mother and the pups (Agostino and Henning, 1982; Wysocki and Segal, 1972; Blake and Henning, 1983). The fifteenth day was chosen for the initiation of experimental treatment because available evidence suggests that in the rat the fetal thyroid is not funtionally mature before eighteenth or nineteenth day of gestation and that during the first two trimesters of the gestational period maternal thyroxin probably does not pass the placental barrier (Hamburgh et al. 1962).

Litters from both group of rats were taken and killed by decapitation on days 1, 5, 15, 25 after birth. Adult rats were similarly killed. Animals were weighed before being killed and following decapitation , the liver, heart and brain were removed and weighed. Phospholipid content of these tissues was determined as was the fatty acid composition of these phospholipids. Following decapitation blood was collected from all animals in heparinized microhematocrit tubes. Upon centrifugation the plasma was frozen (at- 80° C)

and kept for later determination of thyroxine and triiodothyronine.

3.3 Radioimmunoassay of Thyroid Hormones

The plasma thyroxine and triiodothyronine concentrations were determined with the use of the respective Amerlex Radioimmunoassay Kits (Amersham Ltd). All standards were measured in triplicate whilst all plasma samples were measured in duplicate. These kits have previously been shown to be satisfactory for use with rat plasma (Hulbert and Augee, 1982).

3.4 Lipid Separation And Phospholipid Analysis

Lipids were extracted from liver, heart and brain tissues using the procedure of Folch et al. (1957) then dried under nitrogen and redissolved in chloroform. Neutral lipids and phospholipids were separated by silicic acid column chromatography (Sil LC, 325 mesh). Neutral lipids were eluted with 6 column volume of chloroform, and phospholipids were eluted with 3 column volume of chloroform : methanol (1 : 2) followed by 6 column volume of methanol (Rovinski and Hosein,

1983). In order to determine the phospholipid content of the tissues a known aliquot of the total phospholipid fraction was digested according to the method of Harris and Popat (1954). Phosphorous analysis confirmed that the phospholipids were exclusively confined to the fraction eluted with methanol. Also the absence of neutral lipid in the phospholipid fraction was confirmed by thin layer chromatography (Christie, 1982).

3.5 Gas Liquid Chromatography

Methyl esters of total phospholipid fatty acids were prepared by transmethylation with 20% Boron trifluoride in methanol at 75°C for 60 minutes (McMurchie and Raison, 1975). The methyl esters were extracted with petroleum ether (b.p. 40-60°C) and further purified by column chromatography using hydrated florosil (Florosil +7% H_2O), the pure methyl esters were eluted with 4.5 ml of 5% diethyl ether in petroleum ether. Quantitative gas chromatography was performed using a Packard model 427 gas chromatograph. Fatty acid methyl esters were separated by gas liquid chromatography on an open tubular capillary column coated with FFAP (0.22 mm I.D, 0.32 mm O.D, 25 meter long), using a flame ionization detector

(Bourre et al. 1984). The flame ionization detector and the injector were maintained at 260°C and 220°C respectively. The column temperature was maintained at 170°C for 20 minutes, after which a programmed temperature rise of 1° C per minute was initiated until the column temperature was 195°C. The column temperature was then maintained at 195°C for 15 minutes.

Identification of fatty acids methyl ester was achieved by reference to the retention times of known standards (Sigma Chemicals and Nu Chek Prep Inc) injected into the gas chromatograph under the same conditions. Initially some traces were planimetered to determine the mole percent fatty acid composition. Later traces were integrated with the use of Shimadzu C-R 3A chromatopac integrator. Some samples were subjected to both methods of analysis and there was close agreement between the values obtained by either method.

3.6 Statistics Differences of the means were established by student's t-test.

CHAPTER 4

RESULTS

4.1 Plasma Thyroxine and Triiodothyronine Concentrations of Euthyroid and Hypothyroid Rats During Development.

Plasma thyroxine (T_4) and triiodothyronine (T_3) concentrations of euthyroid and hypothyroid rats of various ages are shown in table 2 and figures 4 and 5. Figure 4 illustrates that the serum T_4 concentration is low at 5 days of age and then rises to peak values at days 15 followed by a decline as adolescence is reached. Plasma T_3 concentrations are low at 5 days of age and then increase with age but here also values reach a higher value on day 25. This is followed by a decrease when adulthood is reached. These results are similar with the results obtained by Walker et al. (1980) who measured thyroid hormone concentrations during postnatal development in the rat. It can be seen that at all ages up to 25 days, the provision of PTU in the drinking water resulted in significantly reduced levels of both thyroid hormones compared to those rats not given PTU in their

TABLE 2 : Plasma thyroxine (T_4) and triiodothyronine (T_3) concentration (nmol/lit) of euthyroid and hypothyroid rats of various ages.

Iodotyrosine	Age (Days)				
	1	5	15	25	Adult
T_4 (E)	43.4 ± 8.8	37.1 ± 2.3*	133.9 ± 6.6	106.6 ± 7.1	54.5 ± 5.8*
T_4 (H)	1.1 ± 0.4*	2.8 ± 0.8	3.7 ± 0.3	28.8 ± 2.1	36.1 ± 3.4
Signif. of difference	P<0.005	P<0.001	P<0.001	P<0.001	P<0.02
T_3 (E)	0.0	0.6 ± 0.1#	0.9 ± 0.1*	1.2 ± 0.1	0.6 ± 0.2
T_3 (H)	0.0	0.3 ± 0	0.2 ± 0.1	0.2 ± 0.0*	0.3 ± 0.1
Signif. of difference	-	P<0.05	P<0.001	P<0.001	N.S

Values are means ± S.E.M. E,euthyroid; H,hypothyroid;
n=6 , except *(n=5), #(n=4). N.S. not significant.

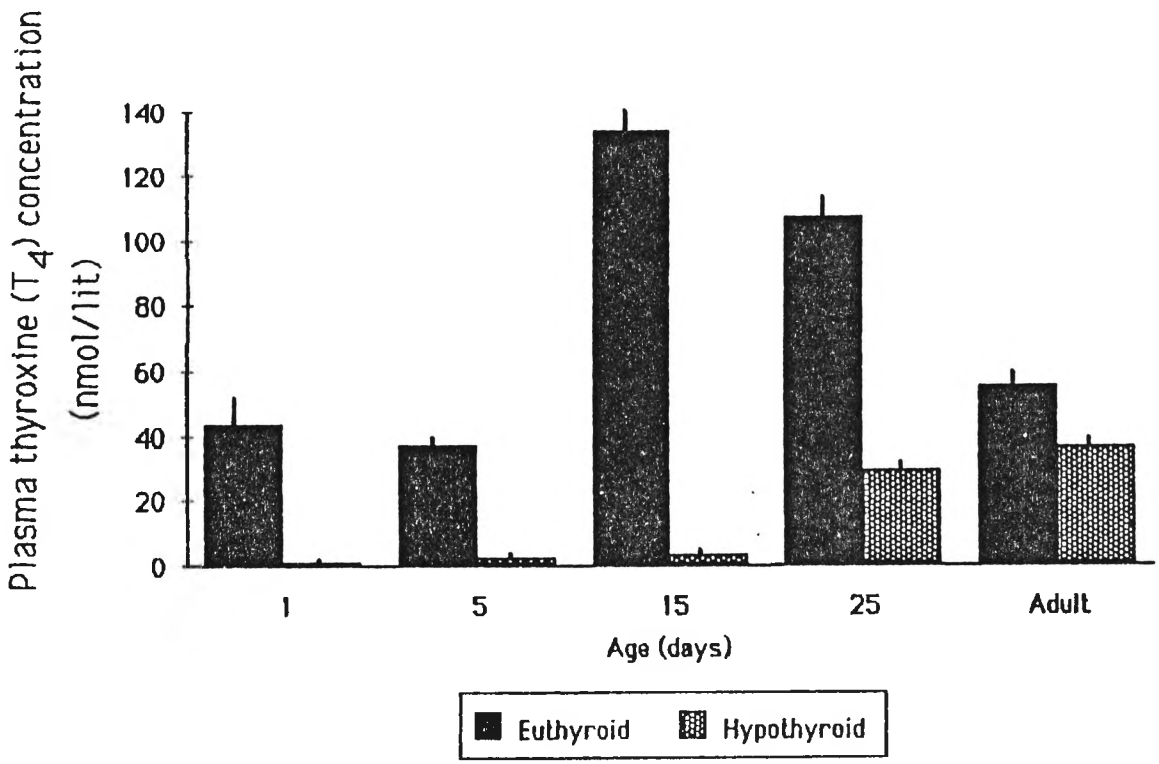

Figure 4 : Plasma thyroxine concentration of euthyroid and hypothyroid rats of various ages.

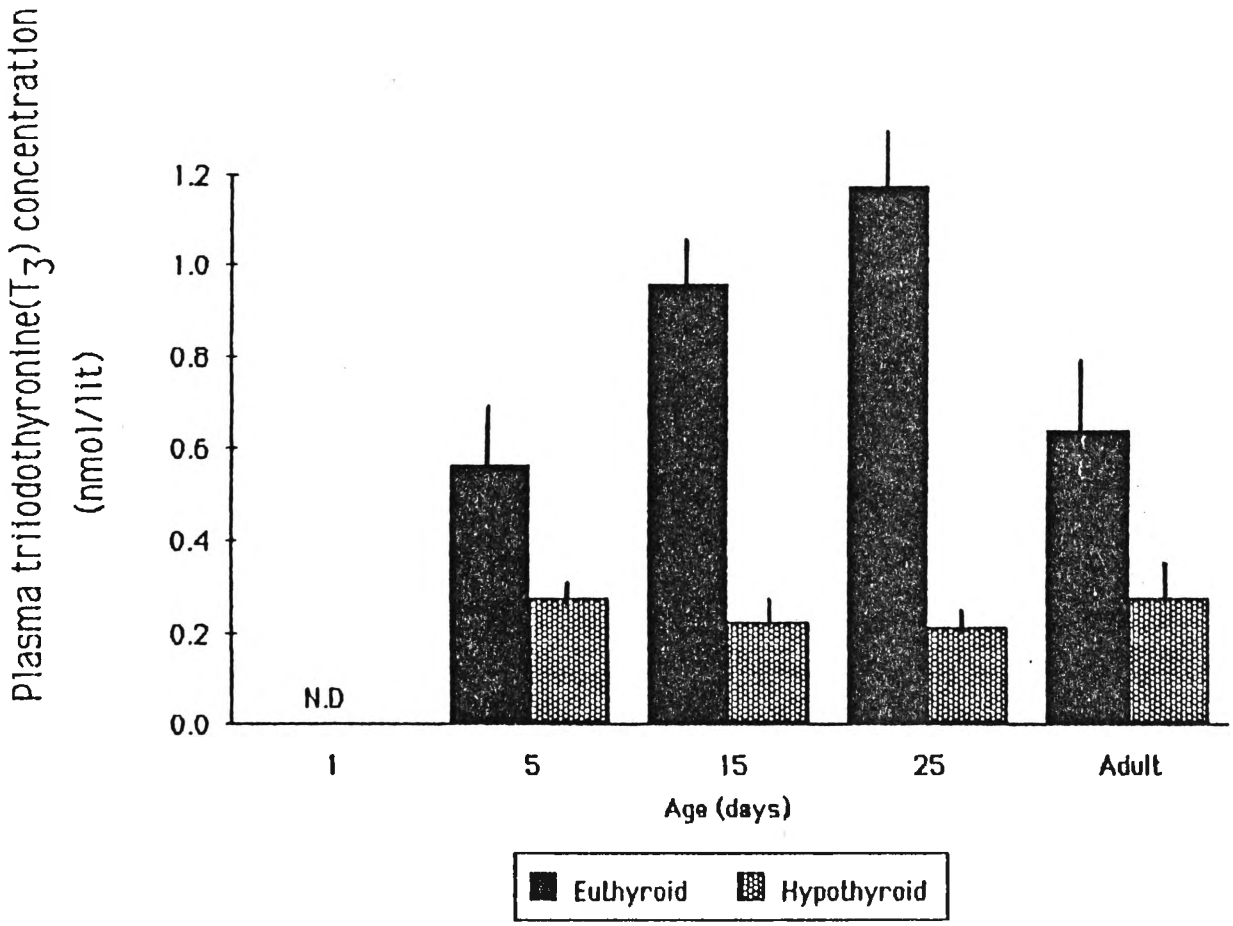

Figure 5 : Plasma triiodothyronine concentration of euthyroid and hypothyroid rats of various ages.
N.D. Not detecttable.

water. This confirms that PTU administration resulted in a hypothyroid condition. An interesting feature of these results is the fact that hypothyroid rats gradually increased their plasma level of thyroxine throughout development in spite of the continued exposure to PTU.

4.2 Body Weight of Euthyroid and Hypothyroid Rats During Development.

The body weight of both euthyroid and hypothyroid rats of various ages is shown in table 3 and figure 6. The result shows that in control rats the body weight increases with age until adulthood is reached. Hypothyroid rats show no difference to euthyroid rats up to day 15, however, after day 15 there is an increasing difference between the euthyroid and hypothyroid young. This result agrees with that obtained by Astwood et al. (1945).

Table 3 : Body weight (g) of euthyroid and hypothyroid rats of various ages.

Treatment	Age (Days)				
	1	5	15	25	Adult
Euthyroid	6.6 ± 0.1	10.2 ± 0.5#	26.2 ± 0.7	52.6 ± 2.4	126.3± 2.1
Hypothyroid	5.4 ± 0.3	10.5 ±0.2	26.5 ± 1.8*	23.5 ± 0.9	30.9 ±2.1
Signif. of difference	P<0.005	N.S	N.S	P<0.001	P<0.001

Values are means ± S.E.M. E,euthyroid; H,hypothyroid

n=6 , except *(n=5), #(n=7). N.S. not significant.

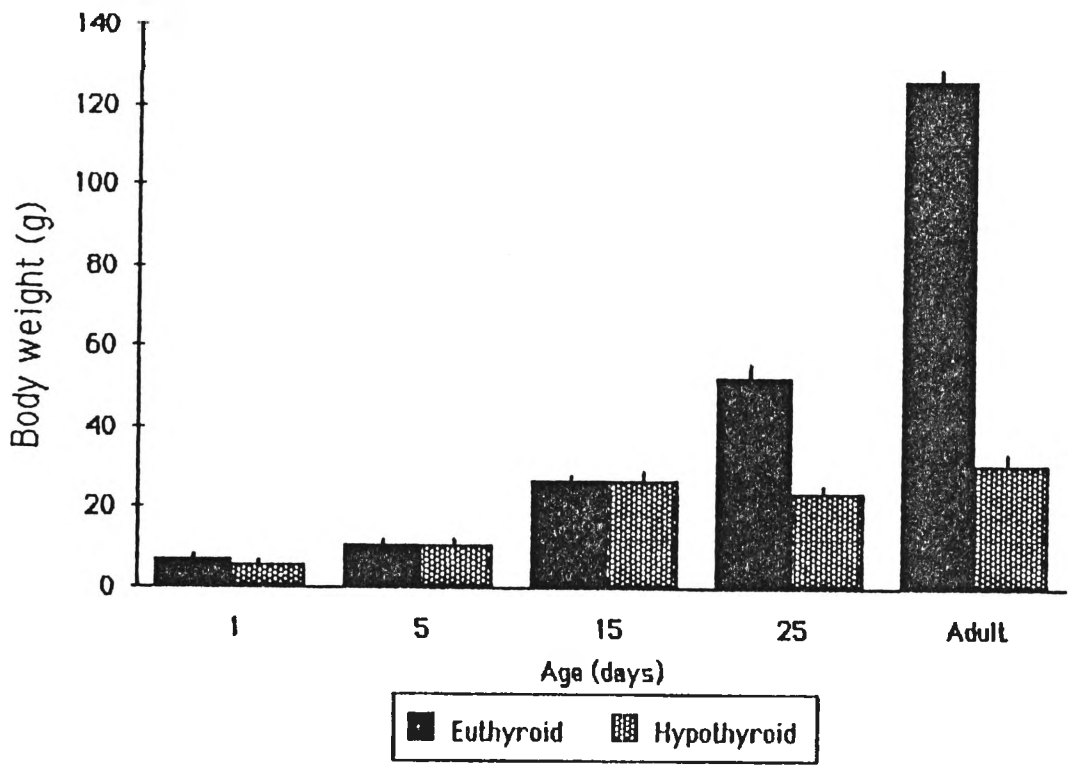

Figure 6 : Body weight of euthyroid and hypothyroid rats of various ages.

4.3 Organ Weight of Euthyroid and Hypothyroid Rats During Development.

Organ weights of both euthyroid and hypothyroid rats of various ages are shown in table 4 and figure 7. In the euthyroid rats liver and heart increase in weight throughout postnatal development. Brain weight, on the other hand, increases up until day 25 and is then followed by a slight decrease once adulthood is reached. In the hypothyroid rats, throughout the postnatal development, organ weights are reduced compared to the euthyroid condition. This result is also similar to that obtained by Scow and Marx (1945) & Scow (1951, 1959). In hypothyroid rats the weights of liver, heart and brain are reduced by 56%, 59%, 36% at 25 days of age and 78%, 72% and 29% for adults when compared to euthyroid rats. The difference in organ weights between hypothyroid and euthyroid rats was small in 1, 5 and 15 day old rats.

Table 4 : Weight (g) of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

Organ	Age (Days)				
	1	5	15	25	Adult
Liver(E)	0.22 ± 0.01	0.30 ± 0.02*	0.81 ± 0.01	2.12±0.10	5.22± 0.18
Liver (H)	0.21 ±0.01	0.32 ± 0.01	1.11 ±0.11	0.93 ± 0.07	1.16 ±0.11
Signif. of difference	N.S	N.S	P<0.05	P<0.001	P<0.001
Heart(E)	0.04 ± 0.00	0.07 ± 0.01*	0.14 ± 0.01	0.24 ± 0.01	0.51 ± 0.01
Heart (H)	0.03 ± 0.00	0.06 ± 0.00	0.11 ±0.01	0.10 ± 0.00	0.14 ± 0.01
Signif. of difference	P<0.05	N.S	N.S	P<0.001	P<0.001
Brain(E)	0.24 ± 0.0	0.48 ± 0.01*	1.15 ±0.06	1.72 ±0.06	1.58 ± 0.02
Brain (H)	0.24± 0.01	0.50 ± 0.01	1.12 ±0.05	1.10 ± 0.01	1.12 ±0.02
Signif. of difference	N.S	N.S	N.S	P<0.001	P<0.001

Values are means ± S.E.M. E,euthyroid; H,hypothyroid.

n=6 , except *(n=7). N.S. not significant.

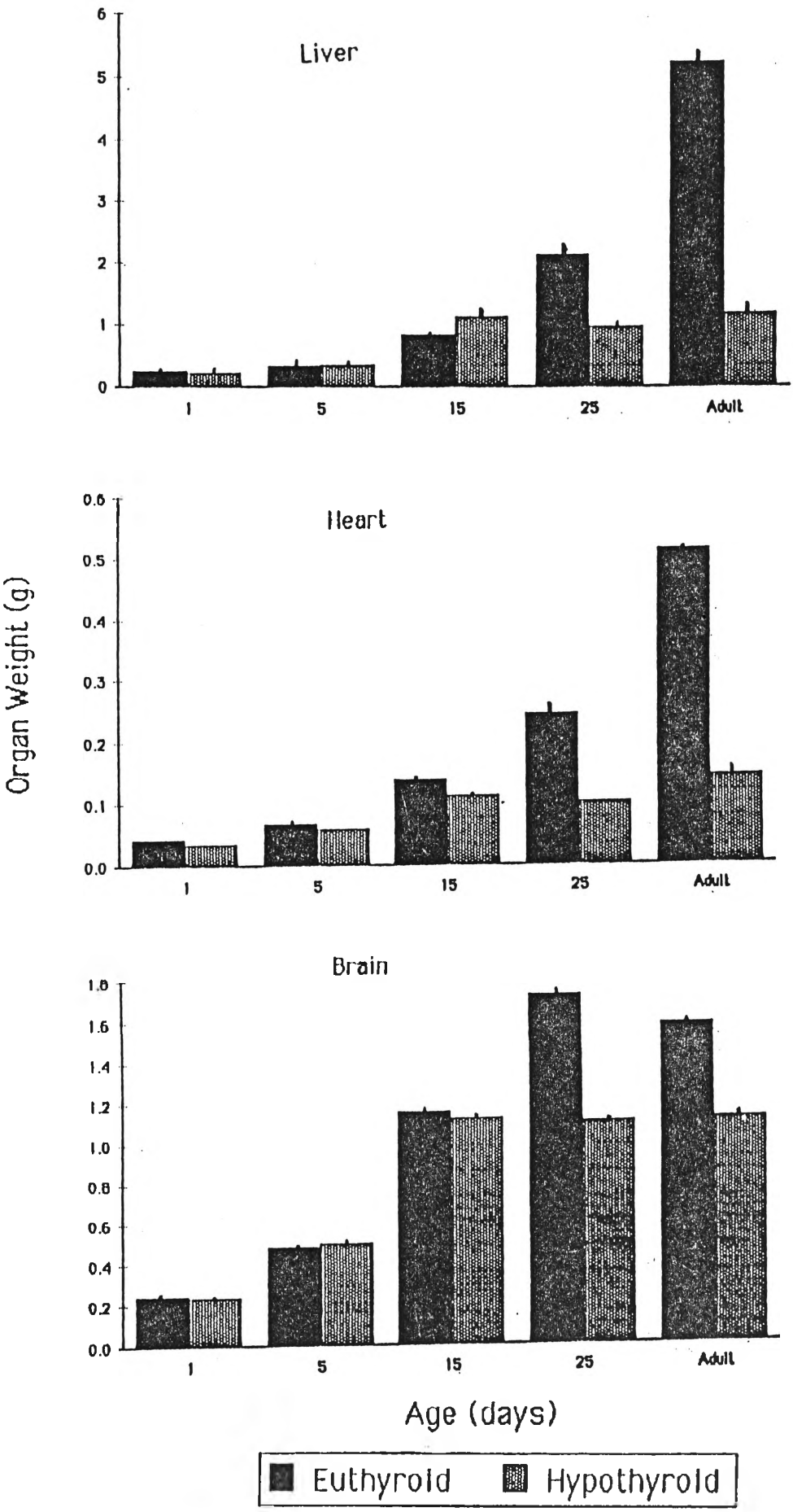

Figure 7 : Organ weights of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

4.4 Phospholipid Content of Liver, Heart and Brain From Euthyroid and Hypothyroid Rats During Development

The phospholipid content of euthyroid and hypothyroid rat tissues of various ages is shown in Table 5 and figure 8. The data for euthyroid rats indicate that in liver and heart phospholipid content increased up to day 15 then decrease to 25 days and again rises until adulthood is reached. In brain, phospholipid content rises continually throughout postnatal development until maturity is reached. These results agree with the result obtained by Davison et. al (1966).

During the first five days of age, in both the liver and heart there is no difference in phospholipid content between euthyroid and hypothyroid rats. However, phospholipid content of the brain is greater in hypothyroid rats when compared to euthyroid rats. In hypothyroid rats the phospholipid content of liver increases throughout 15 days and then remains constant until 25 days of age followed by a decrease once adulthood is reached. Phospholipid content in liver of hypothyroid rats is higher during postnatal development (but not in adult rats) as

Table 5 : Phospholipid content (mg/g tissue weight) of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

Organ	Age (Days)				
	1	5	15	25	Adult
Liver (E)	0.72 ± 0.26	0.82 ± 0.04#	0.93 ± 0.01	0.79 ± 0.02	1.08 ± 0.04
Liver (H)	0.84 ±0.05	0.91 ± 0.04	1.15 ± 0.05	1.14 ± 0.06	0.96 ± 0.07
Signif. of difference	N.S	N.S	P<0.002	P<0.001	N.S
Heart (E)	0.55 ±0.03	0.70 ±0.03#	0.88 ± 0.02	0.74 ±0.06	0.90 ± 0.01
Heart (H)	0.60 ± 0.04	0.64 ± 0.02	0.70 ± 0.04	0.93 ± 0.04	0.95 ± 0.06
Signif. of difference	N.S	N.S	N.S	P<0.05	N.S
Brain (E)	0.56 ± 0.03	0.61 ± 0.03#	1.07 ± 0.04	1.23 ± 0.08	1.44 ± 0.07
Brain (H)	0.75 ±0.02	0.70± 0.03	1.06 ± 0.03	1.41 ± 0.02	1.24± 0.05
Signif. of difference	P<0.001	N.S	N.S	N.S	P<0.05

Values are means ± S.E.M. E,euthyroid; H,hypothyroid;

n=6, except #(n=7). N.S. not significant.

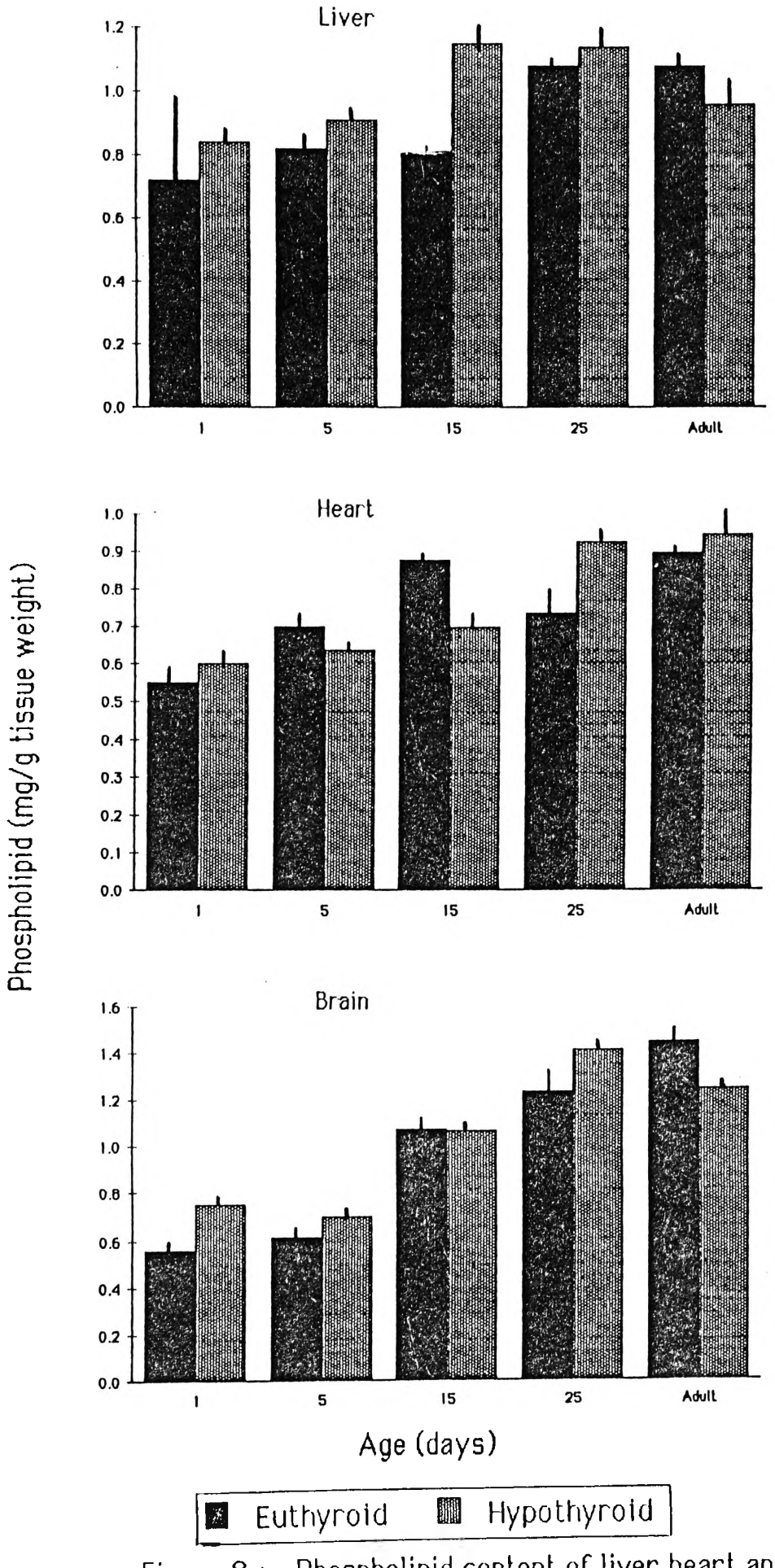

Figure 8 : Phospholipid content of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

compared to euthyroid rats. In hypothyroid rats phospholipid content of the heart increases continually until adulthood is reached and is higher than euthyroid rats in all ages studied. Phospholipid content in brain of hypothyroid rats increases until day 25 as compared with euthyroid rats but during the period of 25 days to maturity the brain phospholipid content decreases.

4.5 Developmental Changes in Fatty Acid Composition of Euthyroid Rats.

4.5.1 Changes in Phospholipid Fatty Acid Composition During Development in Euthyroid Rats.

Changes in the phospholipid fatty acid composition during development of euthyroid rats is shown in Tables 6, 7, 8 and figure 9. The main fatty acids present in phospholipids are palmitic (16 : 0), stearic (18 : 0), oleic (18 : 1), linoleic (18 : 2), arachidonic (20 : 4) and docosahexanoic acid (22 : 6) in all organs studied. In liver, heart and brain phospholipids arachidonic acid is the most abundant fatty acid. Another feature is the large amount of linoleic acid present in liver and heart phospholipids the content of which increased throughout postnatal development. The content of linoleic acid is very low in brain in comparison with liver and heart.

Table 6 : Liver phospholipid fatty acid composition from euthyroid rats of various ages.

Fatty acid	Age (Days)				
	1(*)	5(#)	15	25	Adult(*)
14 : 0	0.5 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	0.42 ± 0	0.4 ± 0.0
16 : 0	11.3 ± 1.3	16.5 ± 1.4	7.6 ± 0.6	10.5 ± 0.7	9.0 ± 0.3
16 : 1	1.1 ± 0.1	1.0 ± 0.4	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
17 : 0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
18 : 0	9.4 ± 1.1	24.8 ± 5.0	7.5 ± 0.6	13.5 ± 0.7	15.4 ± 0.3
18 : 1^{δ}	4.8 ± 0.6	4.7 ± 0.8	2.5 ± 0.3	6.1 ± 0.4	5.7 ± 0.2
18 : 1^{Δ}	1.9 ± 0.2	1.4 ± 0.3	1.0 ± 0.1	1.9 ± 0.1	2.1 ± 0.1
18 : 2	5.8 ± 1.5	5.5 ± 1.3	10.6 ± 1.0	13.9 ± 0.4	14.9 ± 0.4
20 : 0	1.3 ± 0.4	-	3.7 ± 2	-	-
20 : 2	0.9 ± 0.0	-	0.6 ± 0.4	0.6 ± 0.1	0.4 ± 0.0
20 : 3	0.6 ± 0.2	0.1 ± 0.1	0.6 ± 0.2	0.7 ± 0.1	1.0 ± 0.1
20 : 4	35.6 ± 1.6	29.8 ± 3.3	31.5 ± 1.3	33.3 ± 1.1	37.3 ± 0.8
20 : 5	-	-	0.5 ± 0.4	0.3 ± 0.1	0.4 ± 0.1
22 : 3	3.7 ± 0.3	1.4 ± 0.5	1.7 ± 0.2	3.2 ± 0.1	0.9 ± 0.0
22 : 4	2.3 ± 0.2	1.1 ± 0.3	1.7 ± 0.2	1.7 ± 0.1	0.9 ± 0.1
22 : 5	3.4 ± 0.5	1.7 ± 0.5	7.5 ± 0.6	3.4 ± 0.4	1.7 ± 0.1
22 : 6	17.2 ± 2.2	9.8 ± 2.9	21.0 ± 0.9	7.1 ± 0.7	7.3 ± 0.2

Data are given as mole percent and values shown are the mean ± S.E.M (n=6), except *(n=5), # (n=7).

The fatty acids are denoted bt the number of carbon atoms in the chain followed by the number of double bonds.

(18 : 1)$^{\delta}$,Oleic acid; (18 : 1)$^{\Delta}$, Cis Vacenic acid.

Table 7 : Heart phospholipid fatty acid composition from euthyroid rats of various ages.

Fatty acid	Age (Days)				
	1(*)	5(#)	15 (*)	25	Adult
14 : 0	0.9 ± 0.2	3.0 ± 0.8	1.5 ± 0.2	0.8 ± 0.2	0.5 ± 0.1
X_1	1.0 ± 0.2	-	1.0 ± 0.3	1.8 ±0.1	1.4 ± 0.1
16 : 0	6.8 ± 1.1	22.0 ± 2.2	9.9 ± 0.8	9.2 ± 0.7	7.6 ± 1.0
16 : 1	0.2 ± 0.2	1.8 ± 0.4	0.4 ± 0.2	0.7 ± 0.1	0.5 ± 0.1
17 : 0	-	0.3 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
X_2	0.3 ± 0.2		0.9 ± 0.3	1.13 ± 0.1	0.9 ± 0.1
X_3	0.3 ± 0.2	-	0.5 ± 0.2	0.6 ± 0.1	0.6 ± 0.0
18 : 0	7.1 ± 1.5	23.6 ± 2.0	11.2 ± 1.4	13.7 ± 1.1	14.1 ± 1.8
18 : 1^{δ}	7.3 ± 1.3	11.4 ± 1.4	4.2 ± 0.5	6.8 ± 0.5	7.5 ± 0.7
18 : 1^{Δ}	3.5 ± 0.6	2.7 ± 0.5	4.1 ± 0.5	3.5 ± 0.3	3.1 ± 0.3
18 : 2	5.0 ± 0.7	5.0 ±0.5	9.0 ± 1.4	19.2 ± 2.2	24.8 ± 1.0
20 : 0	9.8 ± 2.7	0.1 ± 0.1	9.7 ± 7.5	0.1 ± 0.1	-
20 : 2	0.5 ± 0.2	-	0.1 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
20 : 3	0.5 ± 0.2	-	0.7 ± 0.2	0.7 ± 0.1	0.5 ± 0.1
20 : 4	35.0 ± 1.7	22.2 ± 2.7	27.9 ± 2.2	24.0 ± 0.9	25.1 ± 2.1
20 : 5	2.4 ± 0.9	-	0.9 ± 0.9	-	-
22 : 3	3.2 ± 0.1	1.0 ± 0.6	2.7 ± 1.0	2.8 ± 0.3	2.3 ± 0.2
22 : 4	7.6 ± 0.3	0.1 ± 0.1	3.3 ± 0.6	3.3 ± 0.4	2.5 ± 0.4
22 : 5	2.2 ±0.6	0.9 ±0.7	5.6 ± 0.8	3.0 ± 0.3	2.4 ± 0.4
22 : 6	5.6 ± 0.5	1.0 ± 0.4	6.3 ± 1.2	6.5 ± 1.8	6.3 ± 1.3

Data are given as mole percent and values shown are the mean ± S.E.M.

(n=6, except * (n=5), #(n=7).

The fatty acids are denoted bt the number of carbon atoms in the chain followed by the number of double bonds.

(18 : 1)$^{\delta}$,Oleic acid; (18 :1)$^{\Delta}$, Cis Vacenic acid.

X_1, X_2, X_3, are unidentified compounds.

Table 8 : Brain phospholipid fatty acid composition from euthyroid rats of various ages.

Fatty acid	Age (Days)				
	1	5 (#)	15	25	Adult
14 : 0	1.6 ± 0.3	5.7 ± 1.3	0.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.1
X_1	1.8 ± 0.1	-	1.7 ± 0.1	2.1 ± 0.2	1.7 ± 0.1
16 : 0	15.7 ± 1.0	32.7 ± 1.7	10.3 ± 1.2	12.4 ± 1.4	8.2 ± 1.0
16 : 1	2.9 ± 0.2	4.3 ± 0.6	1.4 ± 0.2	1.1 ± 0.2	0.7 ± 0.2
X_2	0.9 ± 0.3	-	1.8 ± 0.3	2.7 ± 0.2	2.7 ± 0.3
X_3	0.2 ± 0.1	-	0.7 ± 0.1	1.1 ± 0.1	1.5 ± 0.1
X_4	0.2 ± 0.1	-	0.4 ± 0.1	0.6 ± 0	1.2 ± 0.1
18 : 0	7.9 ± 0.0	15.3 ± 2.5	7.1 ± 0.8	10.1 ± 0.8	7.6 ± 0.9
18 : 1^{δ}	9.3 ± 0.4	10.3 ± 1.0	7.7 ± 0.6	12.1 ± 0.8	11.2 ± 1.1
18 : 1^{Δ}	2.8 ± 0.1	2.1 ± 0.5	1.9 ± 0.1	3.0 ± 0.2	2.7 ± 0.3
18 : 2	0.9 ± 0.1	2.8 ± 1.1	1.9 ± 0.4	2.9 ± 0.2	3.5 ± 0.2
20 : 0	1.1 ± 0.2		1.6 ± 0.4	0.4 ± 0	1.8 ± 0.2
20 : 2	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0
20 : 3	0.3 ± 0.1	-	0.6 ± 0.2	0.6 ± 0.1	0.4 ± 0.1
20 : 4	21.3 ± 0.5	20.6 ± 2.3	24.5 ± 1.0	19.4 ± 0.8	16.2 ± 0.2
22 : 3	9.5 ± 0.4	1.0 ± 0.5	4.2 ± 0.2	4.4 ± 0.2	3.4 ± 0.2
22 : 4	6.6 ± 0.4	1.1 ± 0.6	7.4 ± 0.4	6.2 ± 0.4	6.5 ± 0.5
22 : 5	-	-	1.5 ± 0.3	0.5 ± 0.1	0.5 ± 0.1
22 : 6	16.9 ± 1.1	3.2 ± 1.6	25.1 ± 2.1	19.5 ± 2.2	28.7 ± 2.9

Data are given as mole percent and values shown are the mean ± S.E.M. (n=6), except # (n=7).

The fatty acids are denoted bt the number of carbon atoms in the chain followed by the number of double bonds.

(18 : 1)$^{\delta}$,Oleic acid; (18 : 1)$^{\Delta}$, Cis Vacenic acid.

X_1, X_2, X_3, X_4 are unidentified compounds.

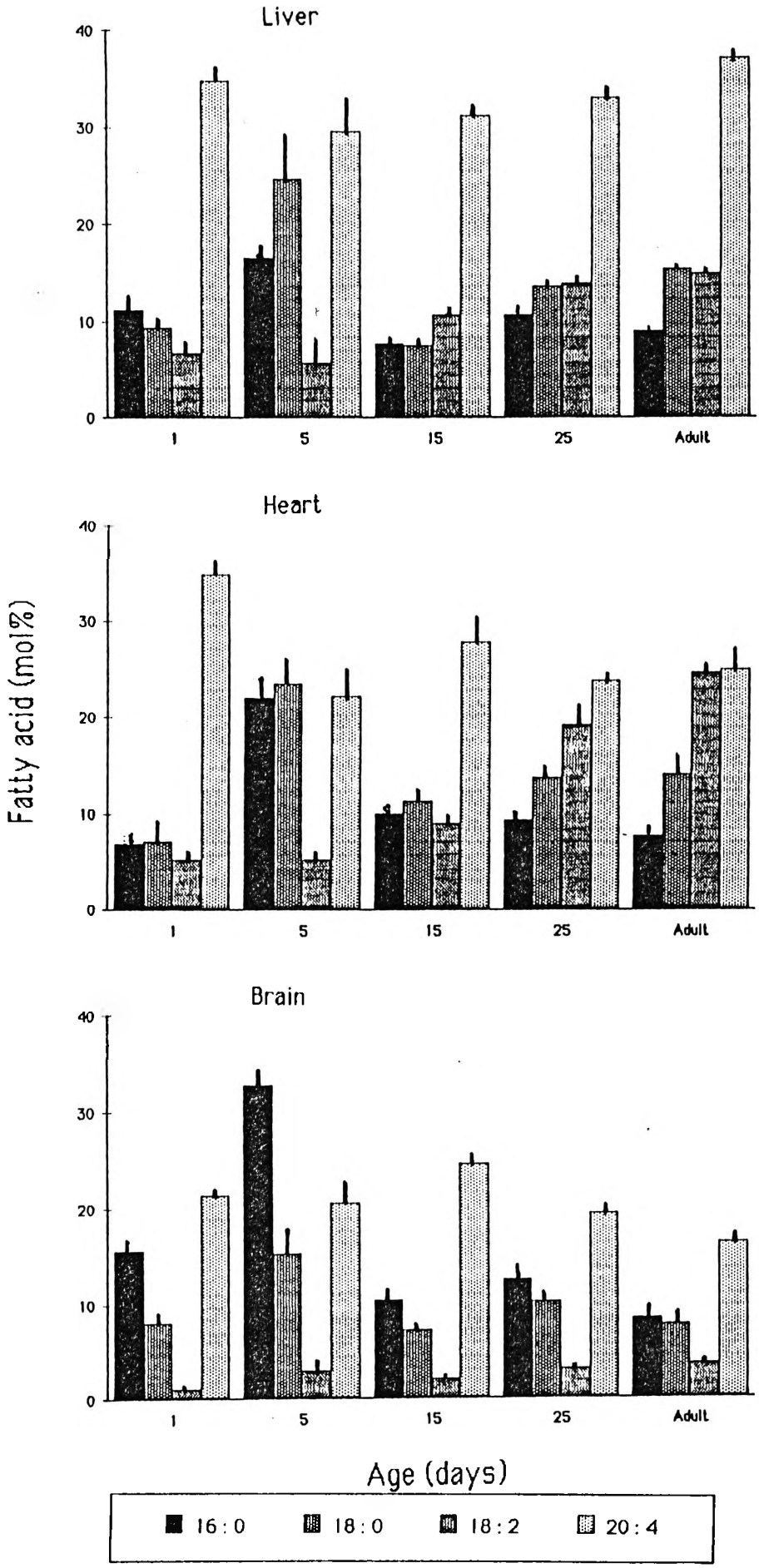

Figure 9 : Fatty acid composition of liver, heart and brain phospholipids from euthyroid rats of various ages.

The palmitic acid (16 : 0) content of phospholipid from all tissues shows a dramatic increase on day 5 and then decreases by day 15. The content of stearic acid (18 : 0) in brain follows a course similar to that of palmitic acid, it being very high on day 5 and then decreasing by day 15. In liver and heart the content of stearic acid is also very high on day 5. It decreases dramatically by day 15 followed by a slight increase until adulthood is reached.

The content of oleic acid (18 :1) in liver, heart and brain is lowest at day 15 followed by an increase once adulthood is reached. The content of linoleic acid (18 : 2) increases with age in liver and heart but in brain the content of linoleic acid is higher on day 5 than on day 15 and is followed by a slight increase once adulthood is reached. The content of arachidonic acid (20 : 4) in liver phospholipid decreases on day 5, then increases with adulthood. In heart and brain the content of 20 : 4 decreases on day 5 followed by an increase by day 15 and a decrease when they become adult. An interesting feature is the high docosahexanoic acid (22 : 6) content in 1 day old rats which decreases dramatically by day 5. This is followed by a dramatic increase in this fatty acid in all organs studied by day 15. It

then remains constant in heart until adulthood is reached. In liver it once again decreases dramatically by day 25 and then remains constant until maturity is reached. In brain the content of 22 : 6 decreases by day 25 followed by an increase in the adult rat.

A significant amount of docosapentaenoic acid (22 : 5) is found in liver and heart in young rats whereas in brain docosatetraenoic acid (22 : 4) and docosatrienoic acid (22 : 3) are more abundant. The unidentified compounds X_1 and X_2 are possibly di methyl acetal of palmitic acid (DMA 16 : 0) and di methyl acetal of stearic acid (DMA 18 : 0) respectively which are derived from plasmalogen phospholipids during the methylation process (Kramer et.al. 1982) and show an increasing content up to day 25 in heart, then decrease once maturity is reached. In brain the content of this DMA 16 : 0 and DMA 18 : 0 increase throughout postnatal development whereas in liver they are absent in all ages studied. No traces of DMA 16 : 0 are found on 5 day old rats. These results are similar with the result obtained by Doviasoba et.al (1961) who measured fatty acid composition in liver phospholipids during development in the rat.

4.5.2 Difference Between Organ Fatty Acid Composition During Development in Euthyroid Rats.

On day 1 phospholipids of the heart are distinctive in that they contain very high proportions of 20 : 0 and very low content of 22 : 6, 16 : 0 and 18 : 0 when compared with liver and brain. Brain phospholipids contain very low amount of 18 : 2, no 22 : 3 and a high content of 16 : 0 and 22 : 5. The liver phospholipids have the same phospholipid fatty acid composition as brain phospholipids except the content of 18 : 2 and 22 : 3 which is higher in liver as compared to brain.

The major difference on day 5 is that liver and heart phospholipids contain less palmitic acid and more stearic acid than brain phospholipids. In brain and heart the content of 16 :1 and 18 : 1 fatty acids is higher than in liver. In brain phospholipids 18 : 2 is about half heart and liver 18 : 2 content. Liver phospholipids contain higher proportion of 22 : 6 than both heart and brain phospholipids.

On day 15 the content of fatty acid differs between the organs studied. In all tissues the content of 20 : 4 remains high at day 15. In liver and brain phospholipids there is also a large

amount of 22 : 6 compared to heart phospholipids. In liver and heart there is considerably more 18 : 2 than found in the brain phospholipids of euthyroid rats of this age. In liver and heart phospholipids there are significant amounts of 20 : 0 and 22 : 5 whilst there are only small amounts of these fatty acids in the brain phospholipids.

On day 25 the content of both palmitic acid (16 : 0) and stearic acid (18 : 0) does not differ much in all tissues studied. The major differences between the tissues at this age seem to be that 18 : 2 content is high in heart and liver phospholipids but low in the brain phospholipids. Liver and heart phospholipids are similar in that they contain significant amounts of 22 : 6 and 22 : 5 roughly in a 2 : 1 proportion, whilst brain phospholipids contain a greater amount of 22 : 6 and virtually no 22 : 5.

Finally, however, in adult rats the content of stearic acid (18 : 0) in brain phospholipids is about that of liver and heart phospholipids. The content of palmitic acid is similar in all organs studied. The major difference between the tissues in adult euthyroid rats is the very large amount of 22 : 6 in the brain. Brain phospholipids have about 4 times the 22 : 6 content than both liver and heart phospholipids. Brain phospholipids also

contain more 22 : 3 and 22 : 4 than do liver and heart phospholipids but they contain very little 22 : 5. The 20 : 4 content of brain phospholipids however is less than that of the other two tissues.

As can be seen from figure 9, phospholipid fatty acid content in 25 day old and adult rats is similar but different between the different tissues. All tissues contain approximately the same amount of 16 : 0 but whereas in liver the relative amounts of 18 : 0 and 18 : 2 are similar to the 16 : 0 content, in heart there are increasing amounts of 18 : 0 and 18 : 2 respectively whilst in the brain phospholipids there are decreasing amounts of 18 : 0 and 18 : 2 compared to the 16 : 0 content.

4.6 Changes in Phospholipid Fatty Acid Composition of Tissues From Hypothyroid Rats During Development.

Changes in phospholipid fatty acid composition of tissues from hypothyroid rats are shown in tables 9, 10 and 11 and figure 10. The fatty acids present in phospholipids of hypothyroid rats are similar to those of euthyroid rats but the

Table 9 : Liver phospholipid fatty acid composition from hypothyroid rats of various ages.

Fatty acid	Age (Days)				
	1	5	15(*)	25	Adult
12 : 0	0.8 ± 1.0	0.2 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	-
14 : 0	1.2 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.1 ± 0.1
X_1	0.6 ± 0.0	0.1 ± 0.1	-	0.1 ± 0.1	-
16 : 0	18.8 ± 1.3	24.0 ± 0.5	20.2 ± 0.6	22.3 ± 1.5	21.4 ± 1.3
16 : 1	2.0 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
17 : 0	0.4 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.4 ± 0.2
18 : 0	15.6 ± 0.7	17.1 ± 0.4	21.7 ± 0.5	22.1 ± 1.4	21.8 ± 0.6
$18 : 1^{\delta}$	11.0 ± 0.4	5.4 ± 0.1	3.7 ± 0.1	3.9 ± 0.2	6.0 ± 0.7
$18 : 1^{\Delta}$	3.01 ± 0.2	1.7 ± 0.0	1.5 ± 0.0	1.2 ± 0.1	1.4 ± 0.1
18 : 2	11.9 ± 0.4	10.6 ± 0.1	13.2 ± 0.3	17.8 ± 1.1	14.2 ± 0.3
20 : 2	0.6 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	-	0.1 ± 0.1
20 : 3	0.7 ± 0.2	0.8 ± 0.0	1.0 ± 0.2	0.3 ± 0.1	0.7 ± 0.2
20 : 4	22.2 ± 1.6	23.5 ± 0.6	25.5 ± 0.6	22.7 ± 1.3	23.0 ± 0.9
22 : 3	1.2 ± 0.1	1.3 ± 0.0	1.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2
22 : 4	1.1 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.1
22 : 5	1.5 ± 0.1	2.0 ± 0.1	2.6 ± 0.1	1.4 ± 0.3	1.8 ± 0.2
22 : 6	7.2 ± 0.5	10.4 ± 0.2	5.6 ± 0.3	6.2 ± 0.9	7.7 ± 1.0

Data are given as mole percent and values shown are the mean ± S.E.M (n=6), except *(n=5).

The fatty acids are denoted bt the number of carbon atoms in the chain followed by the number of double bonds.

$(18 : 1)^{\delta}$,Oleic acid; $(18 : 1)^{\Delta}$, Cis Vacenic acid.

X_1 is unidentified compounds.

Table 10 : Heart phospholipid fatty acid composition from hypothyroid rats of various ages.

Fatty acid	Age (Days)				
	1	5	15	25	Adult
12 : 0	2.4 ± 0.1	1.1 ± 0.3	0.6 ± 0.0	0.5 ± 0.1	–
14 : 0	2.6 ± 0.1	1.8 ± 0.2	1.1 ± 0.1	1.0 ± 0.1	0.1 ± 0.1
X_1	1.5 ± 0.1	-	2.4 ± 0.1	2.8 ± 0.6	1.5 ± 0.2
16 : 0	19.8 ± 0.4	23.5 ± 0.5	17.2 ± 0.4	19.9 ± 1.5	17.5 ± 0.8
16 : 1	1.8 ± 0.1	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
17 : 0	0.8 ± 0.1	-	0.2 ± 0.1	1.5 ± 0.3	0.4 ± 0.1
X_2	0.5 ± 0.1	-	1.0 ± 0.2	0.9 ± 0.2	0.8 ± 0.2
X_3	0.7 ± 0.1	-	0.7 ± 0.1	-	0.4 ± 0.1
18 : 0	17.3 ± 0.5	20.3 ± 0.4	20.7 ± 0.3	22.9 ± 1.8	21.2 ± 0.2
18 : 1^{δ}	19.3 ± 0.5	12.9 ± 0.2	6.0 ± 0.2	5.7 ± 0.5	5.3 ± 0.4
18 : 1^{Δ}	3.9 ± 0.2	3.5 ± 0.2	3.1 ± 0.1	3.3 ± 0.1	3.2 ± 0.1
18 : 2	12.8 ± 0.4	8.0 ± 0.4	8.7 ± 0.2	11.2 ± 0.5	15.4 ± 1.2
20 : 2	0.4 ± 0.1	-	0.3 ± 0.1	-	0.1 ± 0.1
20 : 3	0.5 ± 0.1	-	0.8 ± 0.2	-	0.6 ± 0.1
20 : 4	11.3 ± 0.3	21.6 ± 0.4	27.7 ± 0.4	23.2 ± 2.3	23.3 ± 0.6
22 : 3	0.3 ± 0.1	0.3 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	0.7 ± 0.2
22 : 4	1.7 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	1.5 ± 0.3	1.6 ± 0.2
22 : 5	0.3 ± 0.1	0.9 ± 0.2	2.5 ± 0.1	1.5 ± 0.3	2.8 ± 0.3
22 : 6	1.0 ± 0.1	2.8 ± 0.2	2.9 ± 0.2	2.7 ± 0.6	5.0 ± 0.5

Data are given as mole percent and values shown are the mean ± S.E.M (n=6).

The fatty acids are denoted bt the number of carbon atoms in the chain followed by the number of double bonds.

$(18 : 1)^{\delta}$,Oleic acid; $(18 : 1)^{\Delta}$, Cis Vacenic acid.

X_1, X_2, X_3, are unidentified compounds.

Table 11 : Brain phospholipid fatty acid composition from hypothyroid rats of various ages.

Fatty acid	Age (Days)				
	1	5	15	25	Adult
12 : 0	0.9 ± 0.1	-	0.1 ± 0.0	0.2 ± 0.1	-
14 : 0	1.8 ± 0.1	2.0 ± 0.0	1.5 ± 0.1	0.8 ± 0.1	-
X_1	1.7 ± 0.1	-	2.4 ± 0.2	3.3 ± 0.2	2.7 ± 0.1
16 : 0	21.9 ± 1.4	31.9 ± 0.5	28.8 ± 0.9	29.3 ± 2.3	21.7 ± 0.8
16 : 1	4.3 ± 0.3	3.7 ± 0.2	2.2 ± 0.1	1.2 ± 0.1	0.1 ± 0.1
X_2	1.3 ± 0.1	-	2.0 ± 0.2	3.3 ± 0.1	4.4 ± 0.1
X_3	0.5 ± 0.1	-	0.2 ± 0.1	0.9 ± 0.1	1.6 ± 0.1
X_4	0.5 ± 0.1	-	0.1 ± 0.1	0.4 ± 0.1	1.1 ± 0.1
18 : 0	13.4 ± 0.9	16.3 ± 0.2	16.3 ± 0.5	19.0 ± 0.3	19.2 ± 0.5
$18 : 1^{\delta}$	14.8 ± 0.3	11.9 ± 0.2	11.1 ± 0.3	12.2 ± 0.2	14.6 ± 0.3
$18 : 1^{\Delta}$	3.4 ± 0.1	2.6 ± 0.0	2.4 ± 0.1	2.4 ± 0.1	3.1 ± 0.1
18 : 2	4.8 ± 0.3	1.3 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.0 ± 0.1
20 : 2	0.3 ± 0.1	0.2 ± 0.2	-	-	0.1 ± 0.1
20 : 3	0.4 ± 0.1	1.6 ± 0.9	0.3 ± 0.1	-	0.3 ± 0.1
20 : 4	13.4 ± 1.0	13.5 ± 0.2	15.4 ± 0.6	12.6 ± 0.6	10.9 ± 0.2
22 : 3	5.0 ± 0.4	3.0 ± 0.1	2.4 ± 0.2	1.3 ± 0.3	0.8 ± 0.2
22 : 4	4.3 ± 0.3	3.4 ± 0.1	4.1 ± 0.3	3.2 ± 0.4	3.8 ± 0.1
22 : 5	0.2 ± 0.1	-	-	-	-
22 : 6	7.3 ± 0.7	8.4 ± 0.5	8.5 ± 0.5	8.3 ± 0.8	12.8 ± 0.3

Data are given as mole percent and values shown are the mean ± S.E.M (n=6).

The fatty acids are denoted bt the number of carbon atoms in the chain followed by the number of double bonds.

$(18 : 1)^{\delta}$,Oleic acid; $(18 : 1)^{\Delta}$, Cis Vacenic acid.

X_1, X_2, X_3, X_4 are unidentified compounds.

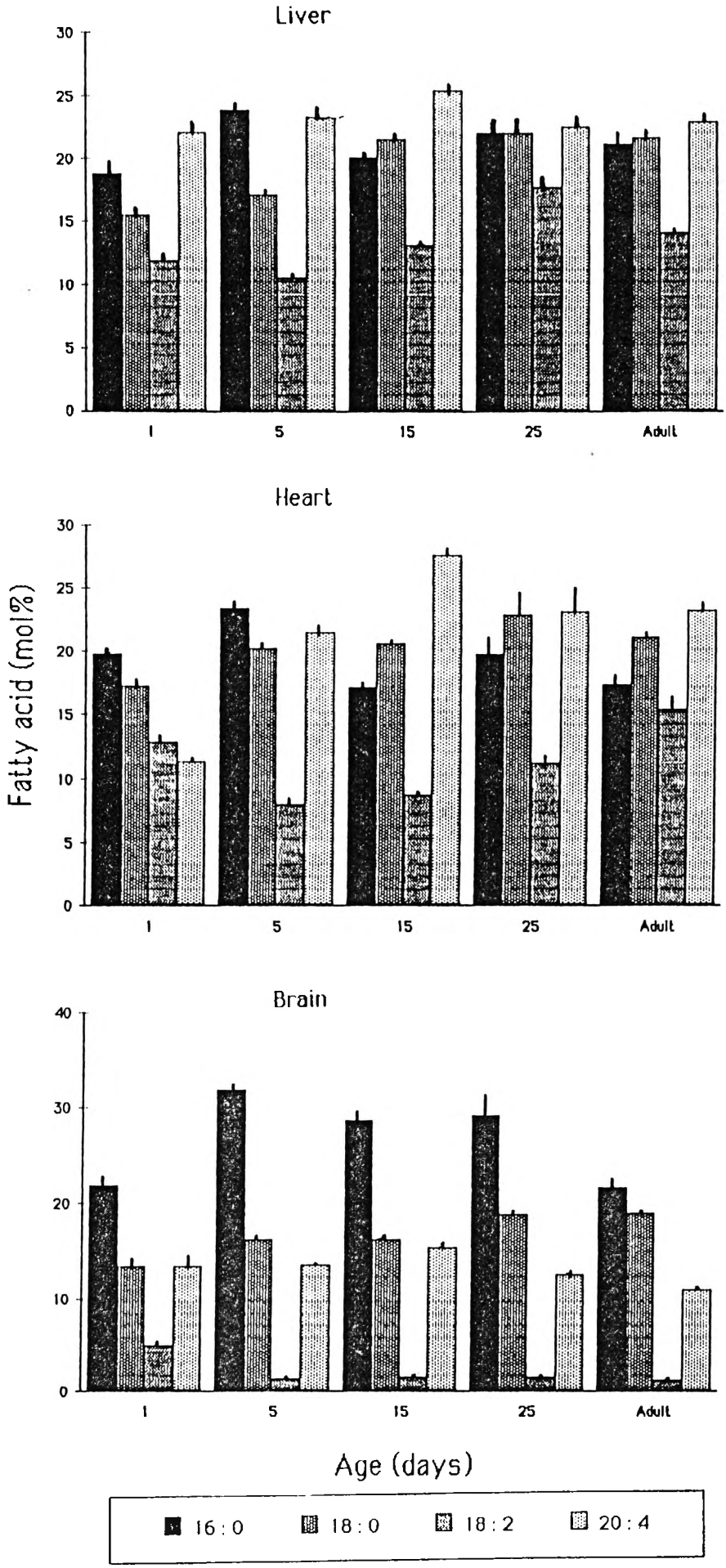

Figure 10 : Fatty acid composition of liver, heart and brain phospholipids from hypothyroid rats of various ages.

relative composition differs greatly. In hypothyroid rats, the liver and heart and brain phospholipid content of palmitic acid is highest at day 5 and there is a slight decrease from this time until they become adult. In hypothyroid rats the content of stearic acid (18 : 0) increases slightly throughout postnatal development in liver, heart and brain phospholipids.

During development in hypothyroid rats the content of oleic acid remains relatively constant in brain phospholipids but decreases on liver and heart phospholipids. In hypothyroid rats the content of linoleic acid (18 : 2) is lowest on day 5 in all organs studied. In heart phospholipids it then increases until maturity is reached but in liver phospholipids it also increases until day 25, then decreases when adulthood is reached. In brain it remains low and relatively constant throughout the post natal period.

In hypothyroid rats heart, liver and brain phospholipid the content of 20 : 4 increases until day 15 followed by a slight decrease once they become adult. In brain and heart phospholipids of hypothyroid rats the content of 22 : 6 increases on day 5 and remains constant until day 25 and shows a further increase once maturity is reached. In liver the phospholipid content of 22 : 6 is greatest on day 5.

4.7 Comparison of Euthyroid and Hypothyroid Rats During Development.

4.7.1 Phospholipids Fatty Acid Composition of Hypothyroid Rats Compared to Euthyroid Rats During Development.

The phospholipid fatty acid composition of liver, heart and brain from euthyroid and hypothyroid rats is summarised in figures 9 & 10 and presented in detail in tables 6 to 11. In this section we are interested in comparing the results for hypothyroid and euthyroid rats. As can be seen from table 12 there are significant differences between euthyroid and hypothyroid rats in the relative content of a large number of fatty acids. This is true for all age groups except the 5 day old rats where there is a much reduced difference between the euthyroid and hypothyroid condition. Hypothyroid rats show : 1) a significant increase in proportion of saturates, due to increase in the proportions of both 16 : 0 and 18 : 0 (figures 11 & 12); 2) a significant change in monoene content (figures 13 & 14); 3) a significant increase in 18 : 2 content in all ages excluding in liver in adults ~~in liver~~, in 15 days rat in heart and 5 and 25 days rat in brain in

Table 12 : Significance of difference of fatty acid composition of different tissues from euthyroid and hypothyroid rats of various ages.

Organ	Fatty acid	1	5	15	25	Adult
	16 : 0	P<0.005	P<0.001	P<0.001	P<0.001	P<0.001
	18 : 0	P<0.001	N.S	P<0.001	P<0.001	P<0.001
	18 : 1	P<0.001	N.S	P<0.01	N.S	P<0.001
	18 : 2	P<0.001	P<0.01	P<0.05	P<0.01	N.S
LIVER	20 :4	P<0.001	N.S	P<0.005	P<0.001	P<0.001
	22 : 3	P<0.001	N.S	N.S	-	P<0.02
	22 : 4	P<0.001	N.S	P<0.02	-	P<0.05
	22 : 6	P<0.001	N.S	P<0.001	N.S	N.S
	16 : 0	P<0.001	N.S	P<0.001	P<0.001	P<0.001
	18 : 0	P<0.001	N.S	P<0.001	P<0.002	P<0.005
	18 : 1	P<0.001	N.S	P<0.01	N.S	P<0.02
	18 : 2	P<0.001	P<0.002	N.S	P<0.01	P<0.001
HEART	20 : 4	P<0.001	N.S	N.S	N.S	N.S
	22 : 3	P<0.001	N.S	N.S	P<0.001	P<0.001
	22 : 4	P<0.001	-	N.S	P<0.01	N.S
	22 : 6	P<0.001	P<0.005	P<0.02	N.S	N.S
	16 : 0	P<0.005	N.S	P<0.001	P<0.001	P<0.001
	18 :0	P<0.001	N.S	P<0.001	P<0.001	P<0.001
	18 : 1	P<0.001	N.S	P<0.001	N.S	P<0.02
	18 : 2	P<0.001	N.S	N.S	P<0.001	P<0.001
BRAIN	20 : 4	P<0.001	P<0.02	P<0.001	P<0.001	P<0.001
	22 : 3	-	P<0.005	P<0.001	P<0.001	P<0.001
	22 : 4	P<0.002	P<0.01	P<0.001	P<0.001	P<0.001
	22 : 6	P<0.001	P<0.02	P<0.001	P<0.001	P<0.001

N.S. Not Significant.

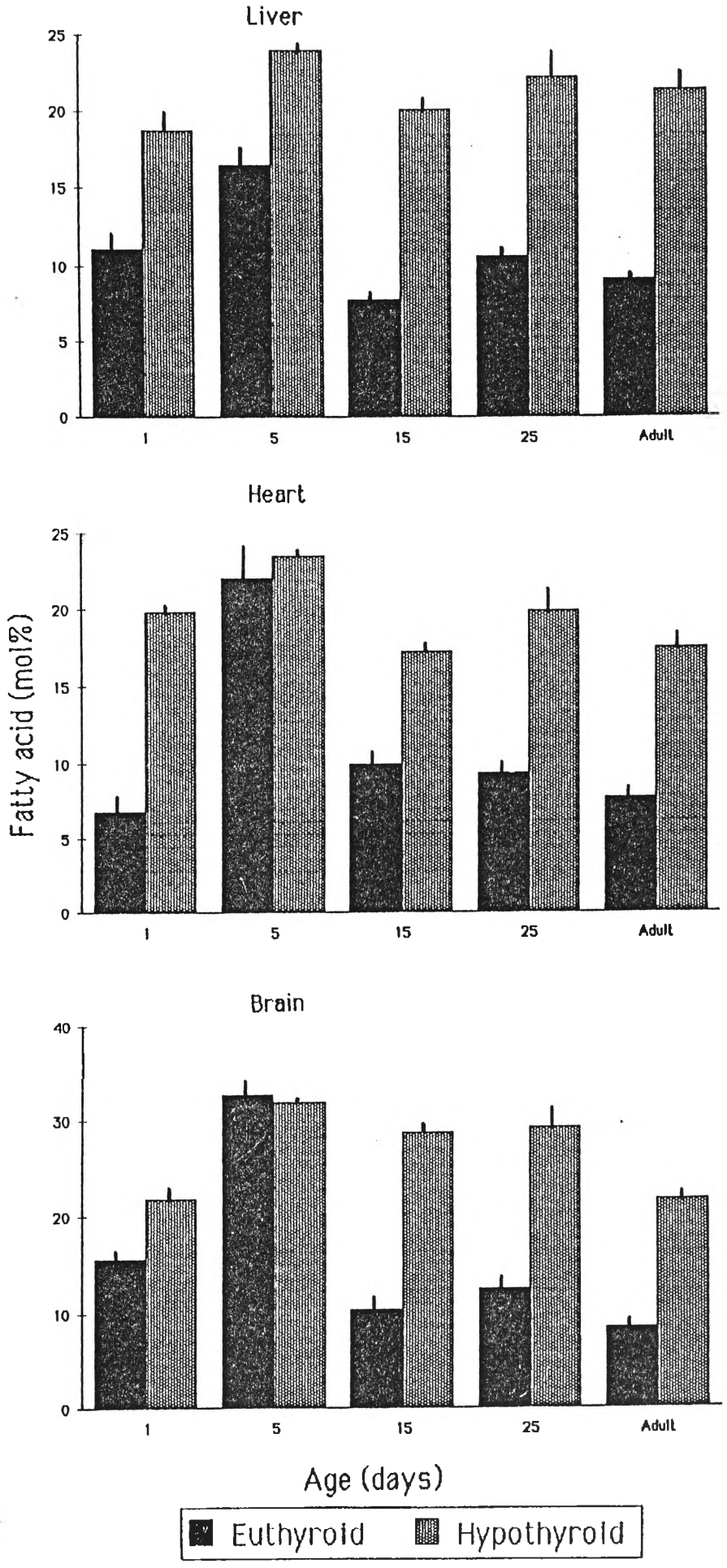

Figure 11 : Palmitic acid (16 : 0) content of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

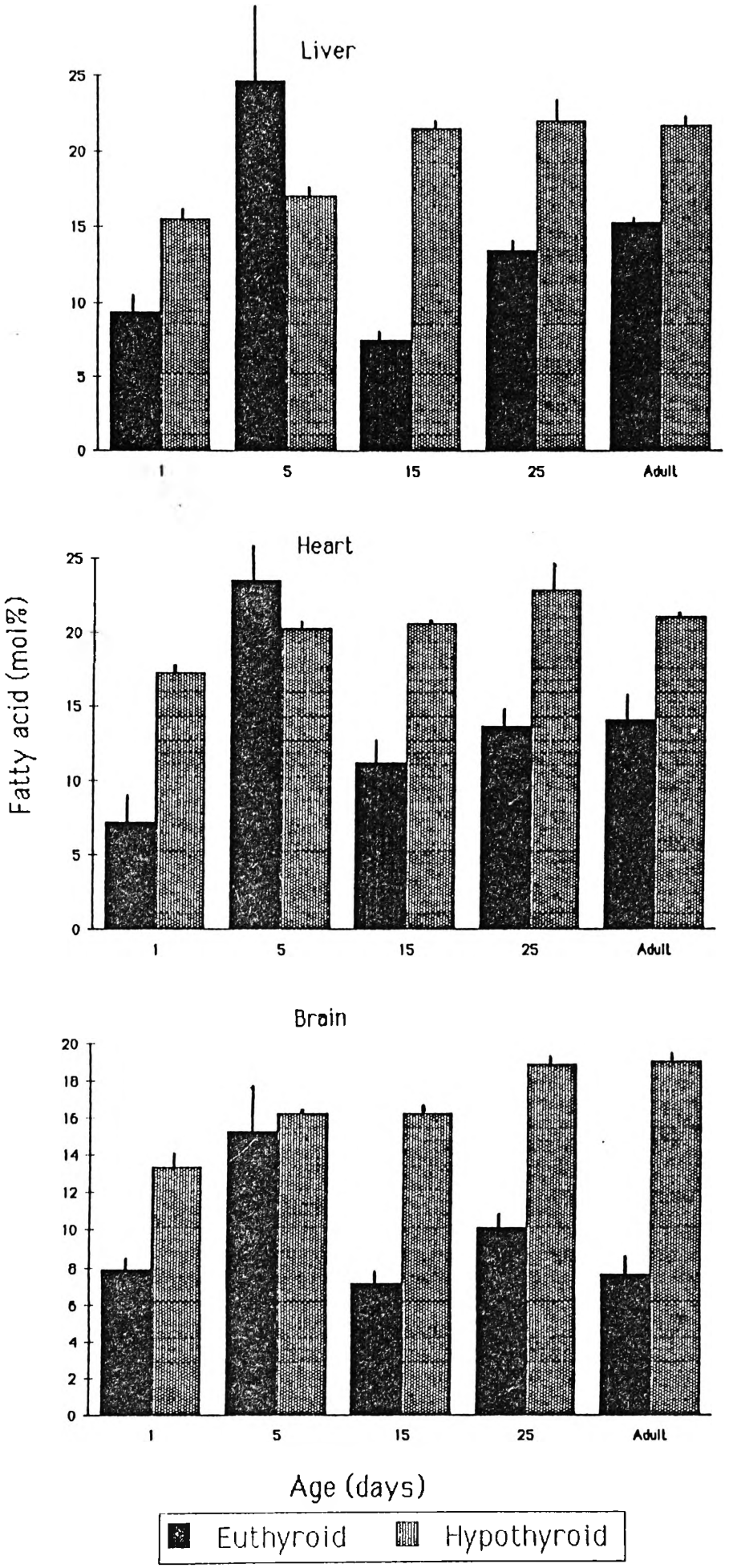

Figure 12 : Stearic acid (18 : 0) content of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

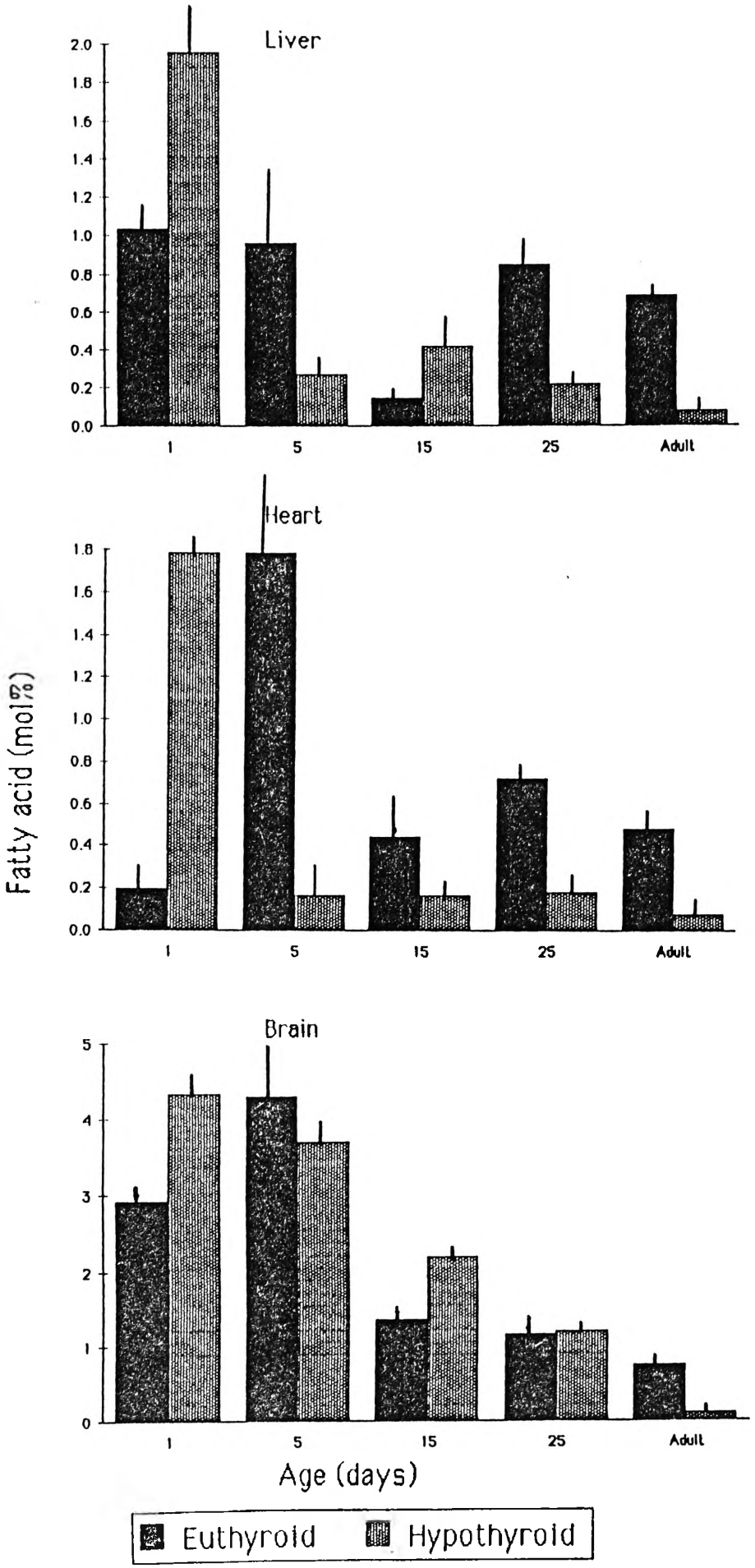

Figure 13 : Palmitoleic acid (16 : 1) content of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

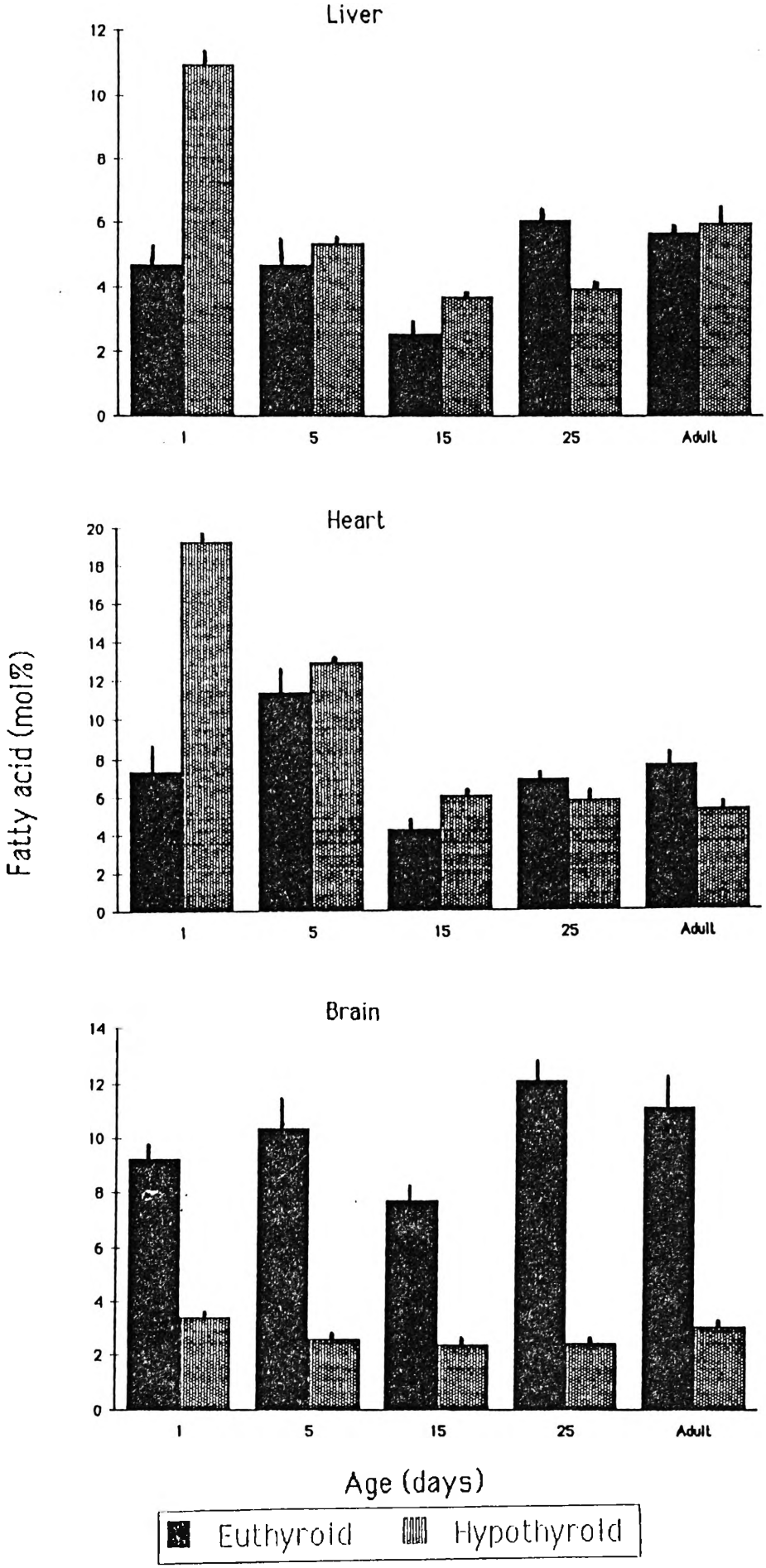

Figure 14 : Oleic acid (18 : 1) content of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

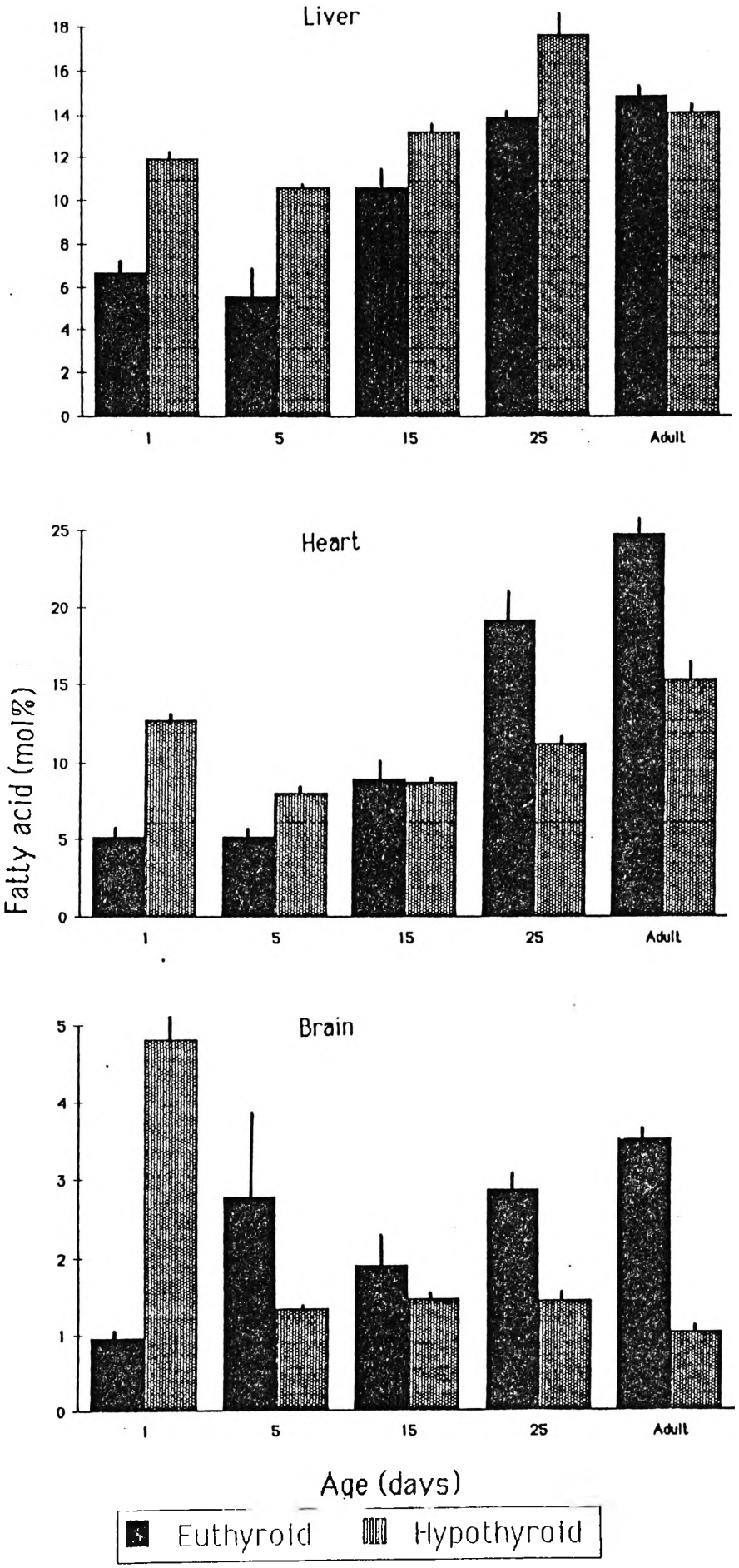

Figure 15 : Linoleic acid (18 : 2) content of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

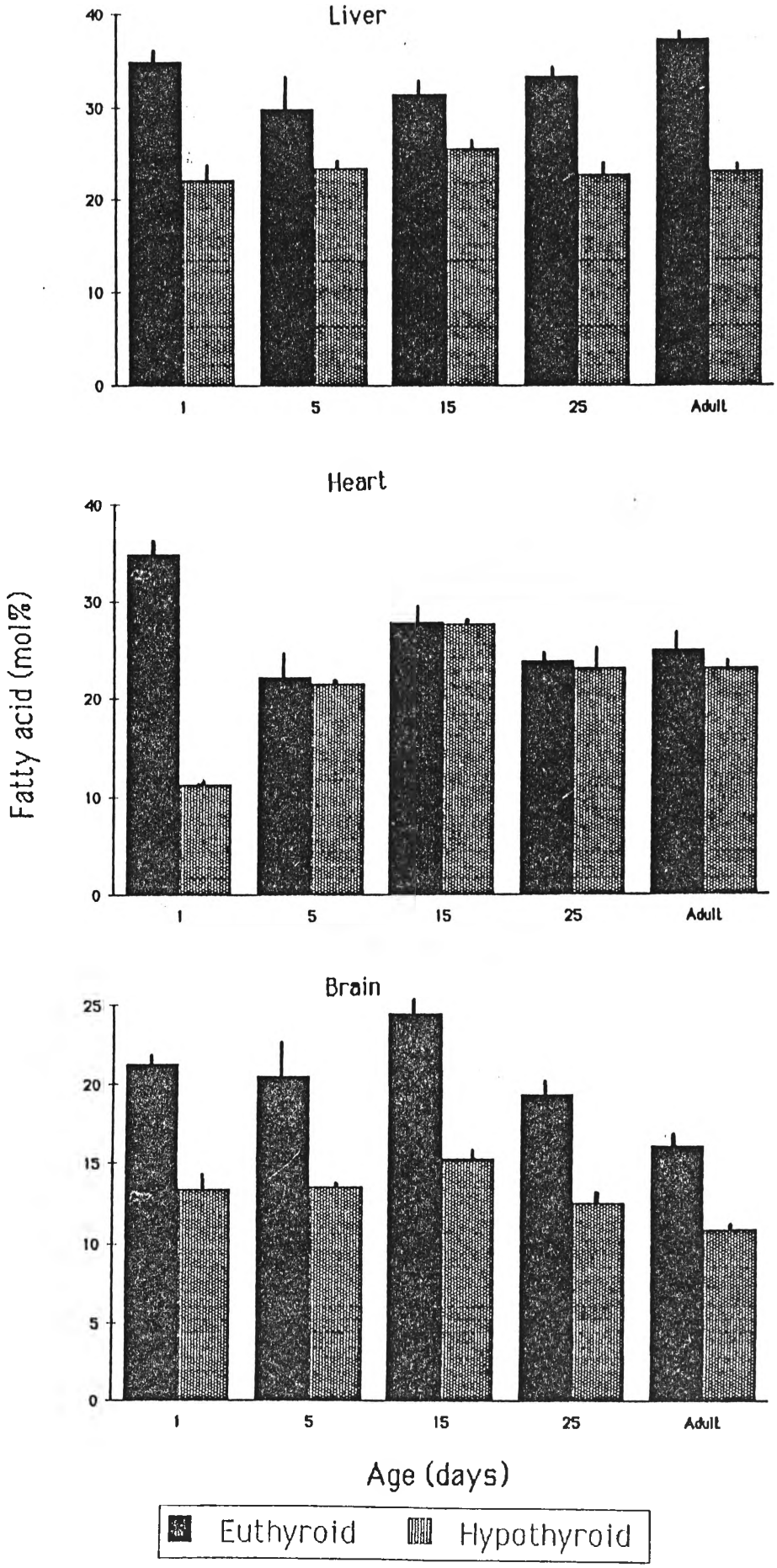

Figure 16 : Arachidonic acid (20 : 4) content of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

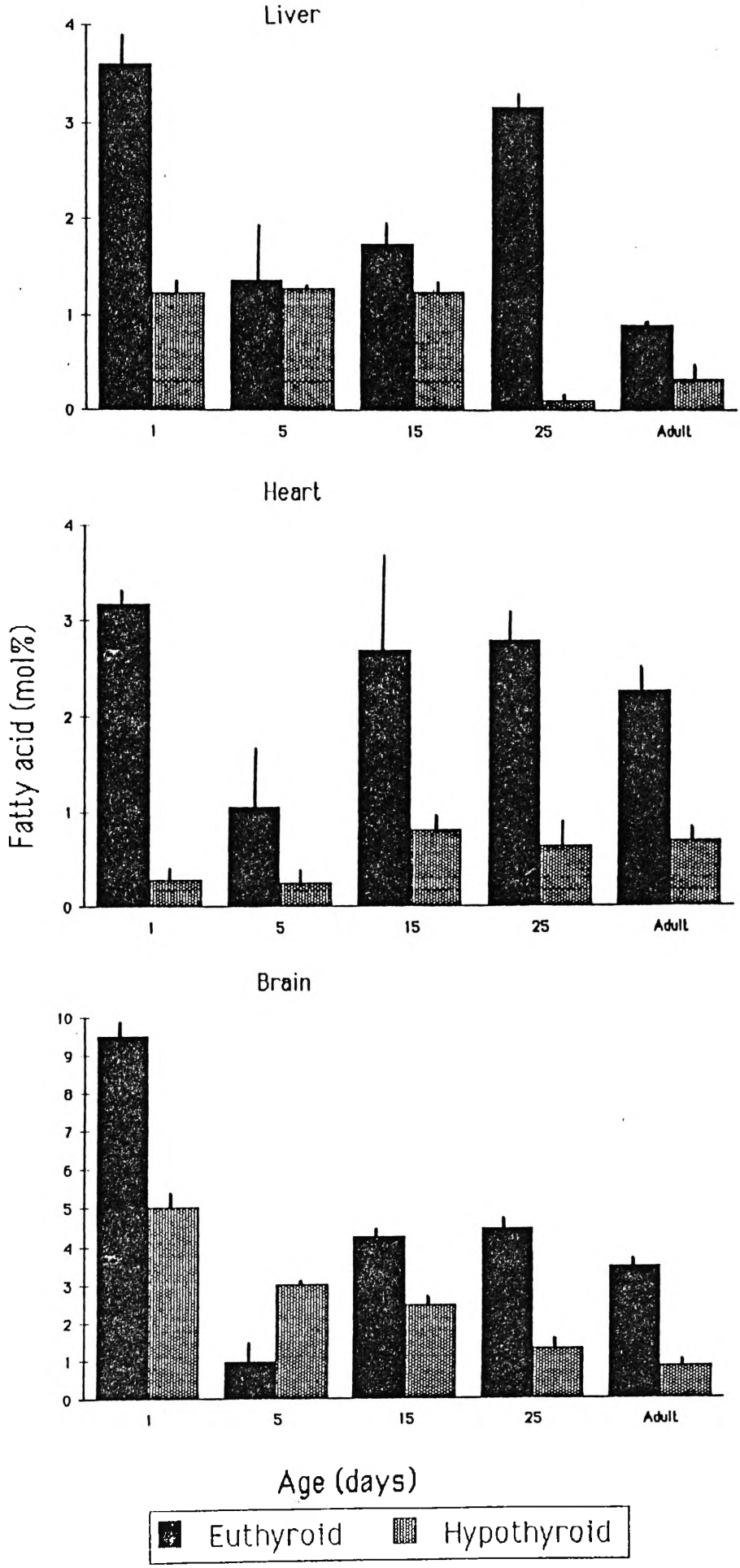

Figure 17 : Docosatrienoic acid (22 : 3) content of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

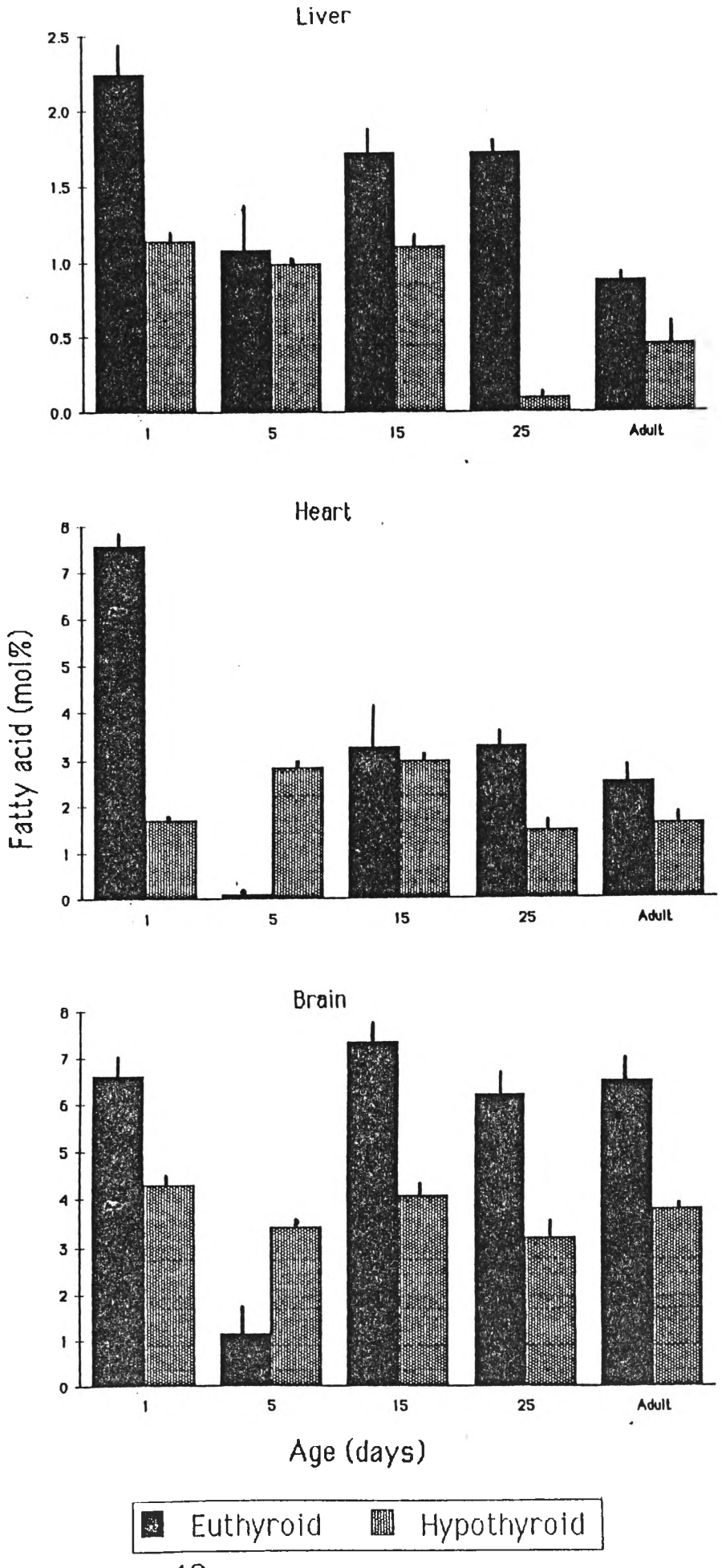

Figure 18 : Docosatetraenoic acid (22 : 4) content of liver,heart and brain from euthyroid and hypothyroid rats of various ages.

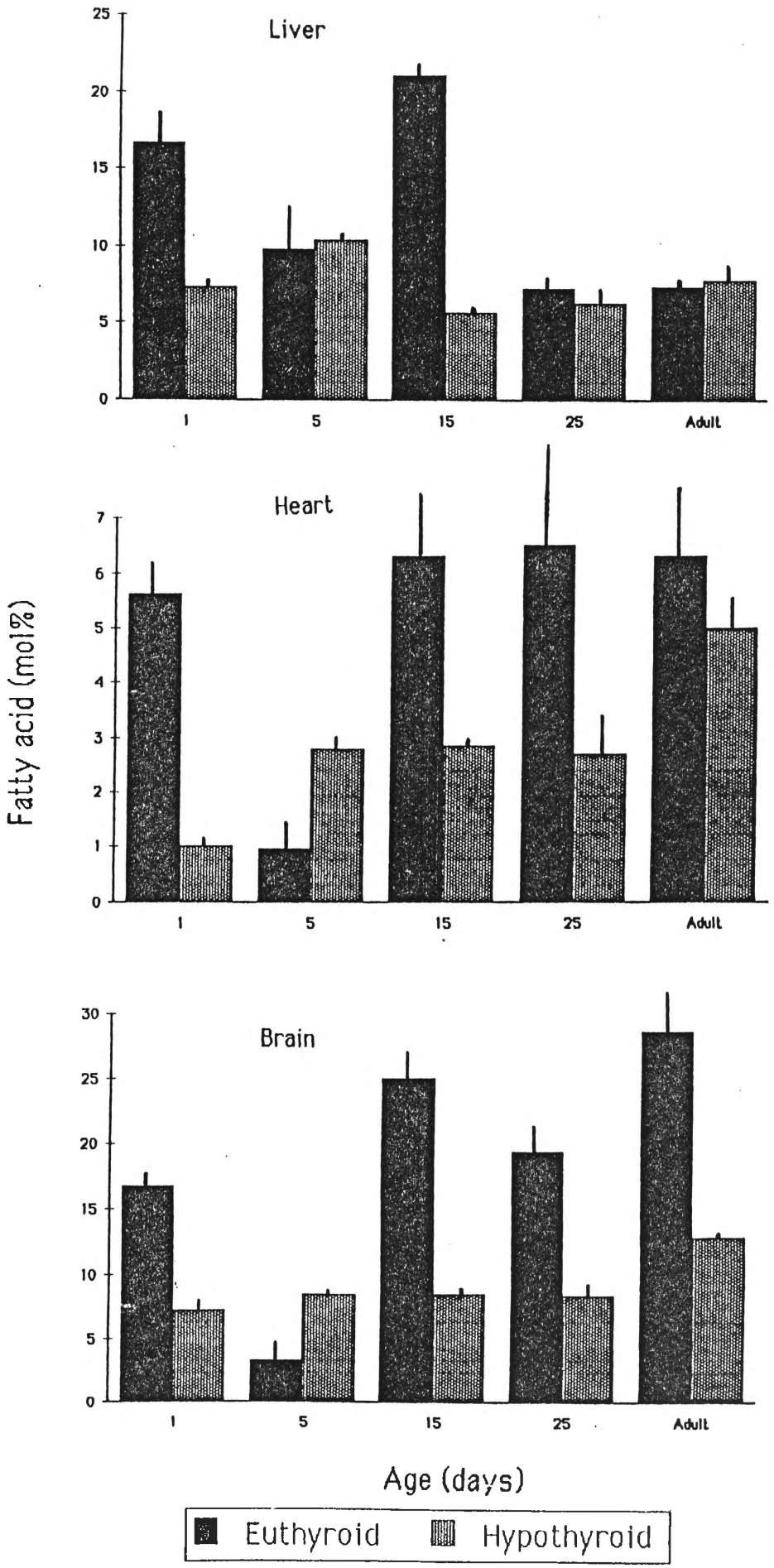

Figure 19 : Docosahexaenoic acid (22 : 6) content of liver,heart and brain from euthyroid and hypothyroid rats of various ages.

in which no significant increase occurs (figure 15); 4) a decrease in the proportion of 20 : 4, 22 : 3, 22 : 4 and 22 : 6 as compared to euthyroid rats (Figures 16 to 19). These changes in fatty acid composition are reflected in lower values of percent unsaturation as well as unsaturation index and ratios of 20:4/18:2.

4.7.2 Percent Unsaturation and Unsaturation Index of Phospholipid Fatty Acids from the Tissues Of Euthyroid and Hypothyroid Rats During Development.

The percent unsaturation and unsaturation index are shown in tables 13,14 and in figures 20, 21. The results for both the percent unsaturation and unsaturation index are similar.

In all tissues in the hypothyroid rats the percent unsaturation of the phospholipid fatty acids remains fairly constant throughout the developmental period. In liver it is in the range of 54-63%, whilst in heart it is 50-58% and in brain phospholipids it is in the range 43-58%.

In all three tissues euthyroid rats, also show similar results. In all three tissues day 1 rats have a percent

Table 13 : Percent unsaturation of phospholipid fatty acids of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

Organ	Age (Days)				
	1	5	15	25	Adult
Liver(E)	76.2 ± 2.6*	56.3 ± 5.8#	79.6 ± 2.4	73.3 ± 1.3	73.5 ± 0.6*
Liver (H)	62.6 ± 1.9	57.3 ± 0.9	56.0 ± 1.0*	53.9 ± 2.7	55.7 ± 1.4
Signif. of difference	P<0.005	N.S	P<0.001	P<0.001	P<0.001
Heart (E)	73.1 ± 2.4*	46.1 ± 2.8#	64.2 ± 5.9*	70.9 ± 1.7	75.7 ± 3.0
Heart (H)	53.5 ± 0.9	52.8 ± 0.6	55.7 ± 0.4	50.1 ± 3.4	57.9 ± 0.4
Signif. of difference	P<0.001	N.S	N.S	P<0.001	P<0.001
Brain (E)	71.0 ± 1.5	45.5 ± 3.2#	76.6 ± 1.9	70.0 ± 2.5	74.4 ± 2.5
Brain (H)	58.2 ± 2.6	49.2 ± 0.5	47.8 ± 1.7	42.6 ± 2.2	47.6 ± 0.8
Signif. of difference	P<0.002	N.S	P<0.001	P<0.001	P<0.001

Values are means ± S.E.M. E,euthyroid; H,hypothyroid;
n=6, except *(n=5), #(n=7). N.S. not significant.

Table 14 : Unsaturation index of phospholipid fatty acids of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

Organ	Age (Days)				
	1	5	15	25	Adult
Liver(E)	300 ± 18*	213 ± 27#	333 ± 9	250 ± 9	252 ± 4*
Liver(H)	191 ± 10	206 ± 4	192 ± 5*	177 ± 10	188 ± 8
Signif. of difference	P<0.001	N.S	P<0.001	P<0.001	P<0.001
Heart(E)	263 ± 15*	127 ± 14#	227 ± 25*	225 ± 11	230 ± 18
Heart(H)	117 ± 2	152 ± 3	184 ± 2	156 ± 16	187 ± 4
Signif. of difference	P<0.001	N.S	N.S	P<0.01	P<0.05
Brain(E)	260 ± 10	143 ± 24#	316 ± 13	259 ± 18	299 ± 21
Brain(H)	164 ± 11	152 ± 2	155 ± 7	136 ± 10	159 ± 3
Signif. of difference	P<0.001	N.S	P<0.001	P<0.001	P<0.001

Values are means ± S.E.M. E,euthyroid; H,hypothyroid;

n=6, except *(n=5), #(n=7). N.S. not significant.

The unsaturation index is Σ(fatty acid mol percent xnumber of unsaturated bonds).

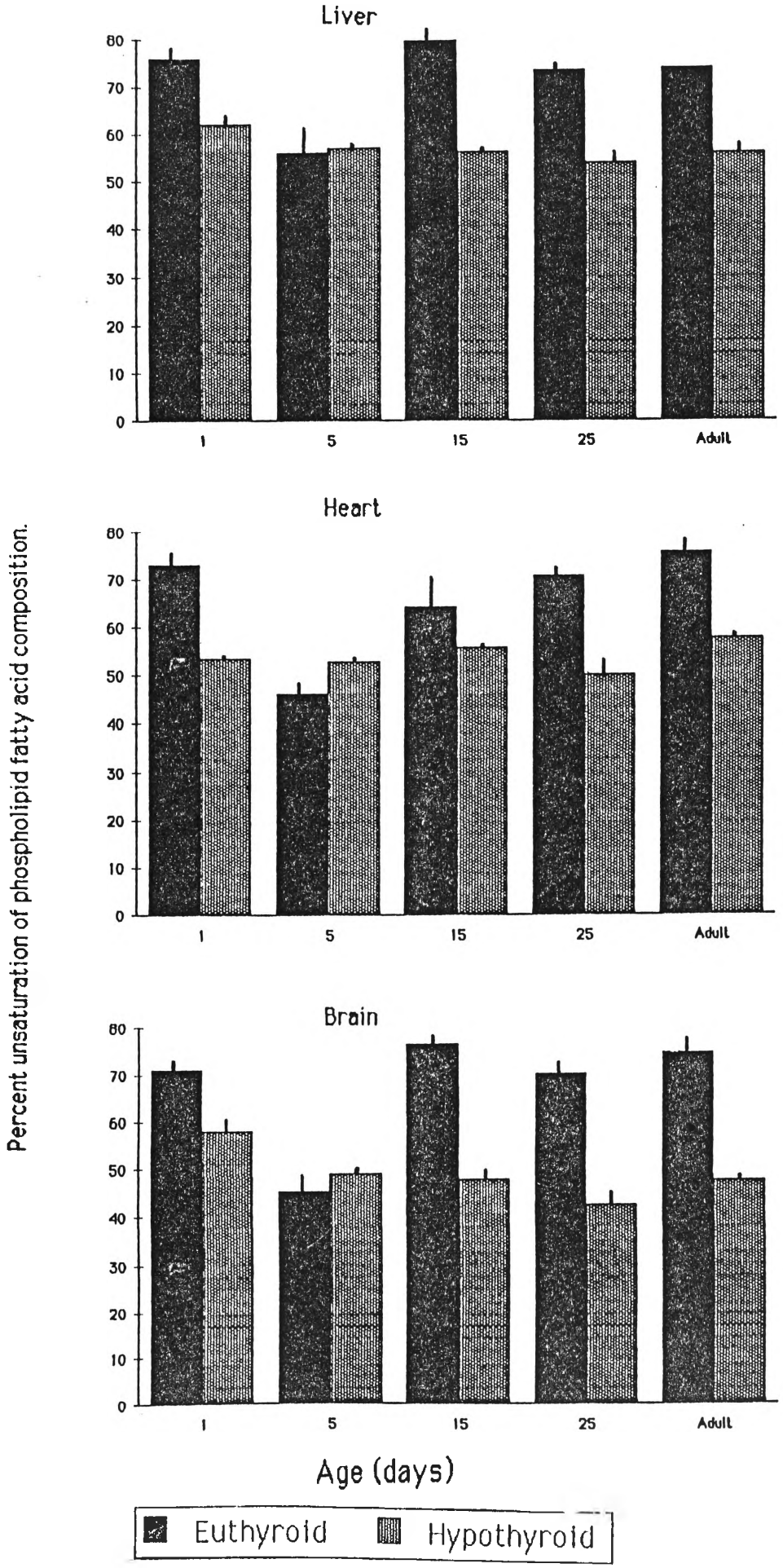

Figure 20 : Percent unsaturation of fatty acid composition of liver,heart and brain from euthyroid and hypothyroid rats of various ages.

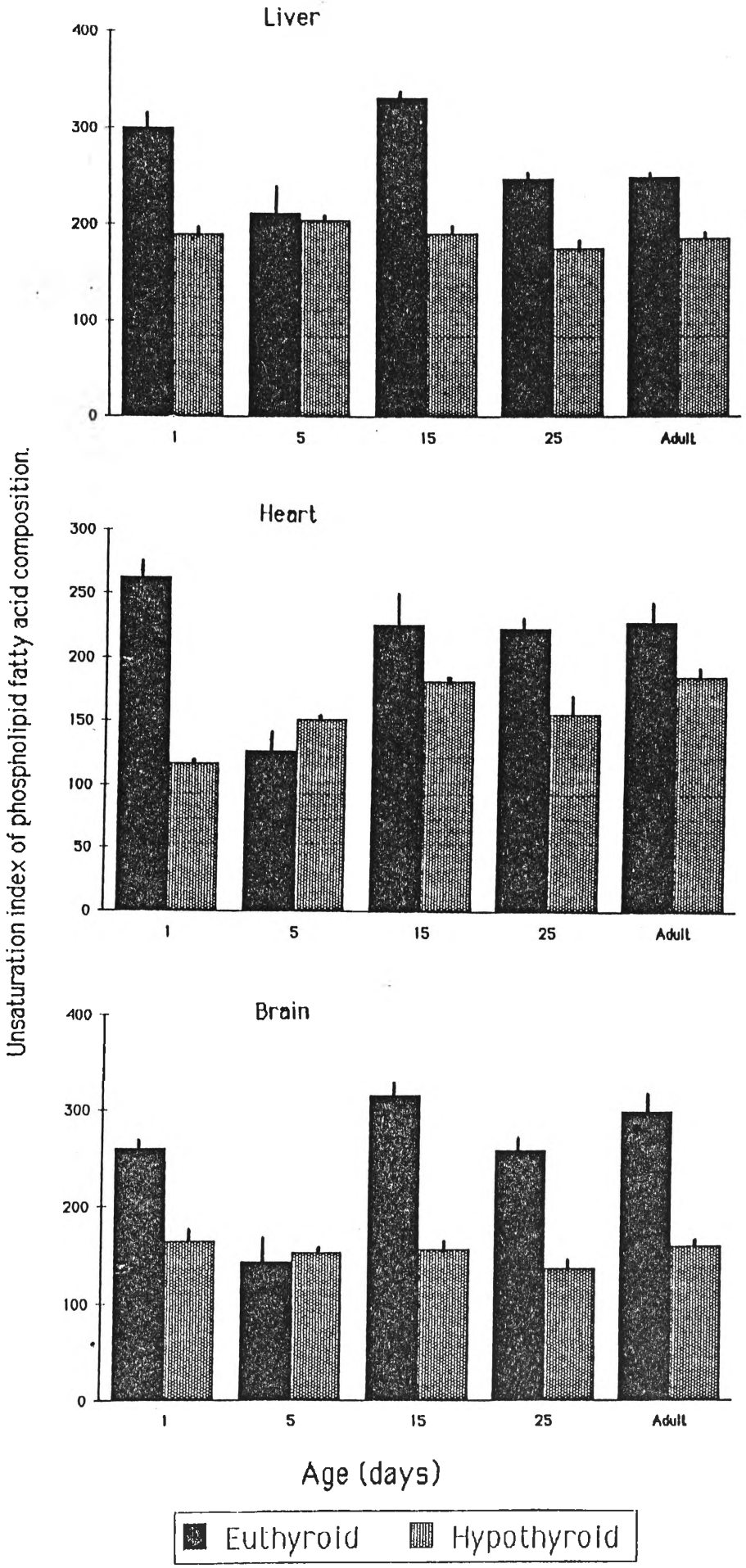

Figure 21 : Unsaturation index of fatty acid composition of liver,heart and brain from euthyroid and hypothyroid rats of various ages.

unsaturation of 71-76% but by day 5 the values drop dramatically to 46-56%, values the same as those found in the hypothyroid group. By day 15 and until adulthood, percent unsaturation values increase to levels similar to those found in one day old rats. Except for 5 day old rats (and heart in 15 day old rats) the tissue phospholipid fatty acids in euthyroid rats have a significantly greater percentage of unsaturated fatty acids.

The unsaturation index results show the same pattern as found for percent unsaturation. In the hypothyroid rat tissues, the unsaturation index is fairly constant throughout the developmental period ranging from 117-187 in the heart to 136-164 in the brain and 177-206 in the liver. In the euthyroid rat the unsaturation index is significantly higher than in the hypothyroid condition except for 5 day old rats (and heart in 15 day old rats) whereas it is similar to the hypothyroid value. In euthyroid rats (omitting day 5 rats) the unsaturation index is 250-333 in the liver, 225-263 in the heart and 259-316 in the brain.

4.7.3 The Ratio of 20:4/18:2 of Phospholipid Fatty Acids from the Tissues of Euthyroid and Hypothyroid Rats During Development.

The ratio of 20:4/18:2 are shown in table 15 and figure 22. The ratio of 20:4/18:2, an indication of desaturase activity, decreases throughout postnatal development in heart and liver but in brain a dramatic decrease occurs on day 5 followed by increase on day 15 and then decreases until adulthood is reached. In brain and heart, the ratio of 20:4/18:2, an indication of $\Delta 5$ desaturase activity is lower until day 15 in hypothyroid rats as compared to euthyroid rats. This then increases until maturity in hypothyroid rats as compared to euthyroid rats.

Table 15 : The ratio of 20:4/18:2 of phospholipid fatty acids of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

Organ	Age (Days)				
	1	5	15	25	Adult
Liver (E)	6.7 ± 1.8*	5.0 ± 1.1	3.1 ± 0.2	2.4 ± 0.2	2.5 ± 0.1*
Liver(H)	1.9 ± 0.1	2.2 ± 0.1	1.9 ± 0.1*	1.3 ± 0.1	1.6 ± 0.1
Signif. of difference	P<0.01	P<0.05	P<0.005	P<0.001	P<0.001
Heart(E)	7.6 ± 1.1*	4.7 ± 0.8#	3.4 ± 0.5*	1.4 ± 0.3	1.0 ± 0.1
Heart(H)	0.9 ± 0.0	2.7 ± 0.1	3.2 ± 0.1	2.1 ± 0.2	1.6 ± 0.2
Signif. of difference	P<0.001	N.S	N.S	N.S	P<0.05
Brain(E)	24.3 ± 3.0	15.1 ± 4.4#	15.1 ± 1.9	7.0 ± 0.6	4.8 ± 0.4
Brain(H)	2.8 ± 0.2	10.2 ± 0.2	10.7 ± 0.3	9.0 ± 0.4	11.0 ± 0.7
Signif. of difference	P<0.001	N.S	P<0.05	P<0.05	P<0.001

Values are means ± S.E.M. E,euthyroid; H,hypothyroid;

n=6 , except *(n=5), #(n=7). N.S. not significant.

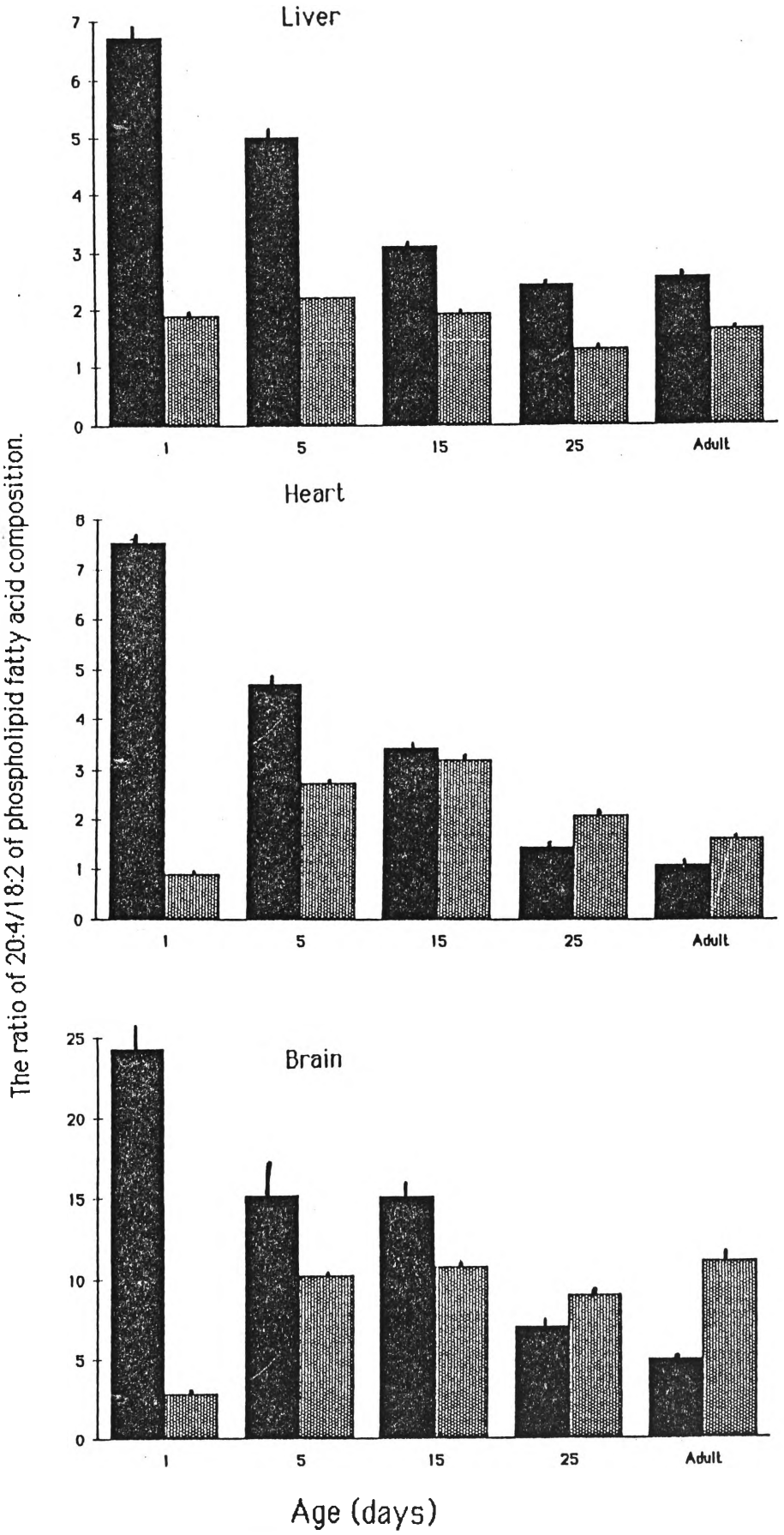

Figure 22 : The ratio of 20 : 4/18 : 2 of fatty acid composition of liver,heart and brain from euthyroid and hypothyroid rats of various ages.

4.7.4 The Mean Carbon Length of Phospholipid Fatty Acids of Liver, Heart and Brain from Euthyroid and Hypothyroid Rats During Development.

The mean carbon length of phospholipid fatty acids of liver, heart and brain from euthyroid and hypothyroid rats of various ages is shown in table 16 and figure 23. The results show that the mean carbon length is similar in all tissues and all ages studied. This result is not in agreement with the results obtained by Shaw and Hoch (1976) and Chen and Hoch (1977) who showed that mean carbon length in fatty acids of membrane was not affected by thyroid state.

however there is a consistent & significant effect of hypothyroidism resulting in a shorter average chain length.

Table 16 : The mean carbon length of phospholipid fatty acids of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

Organ	Age (Days)				
	1	5	15	25	Adult
Liver (E)	19.3 ± 0.2*	18.4 ± 0.2#	19.7 ± 0.1	18.8 ± 0.1	18.8 ± 0.1*
Liver (H)	18.2 ± 0.1	18.5 ± 0.1	18.4 ± 0.1*	18.2 ± 0.1	18.3 ± 0.1
Signif. of difference	P<0.001	N.S	P<0.001	P<0.002	P<0.005
Heart (E)	19.1 ± 0.1*	17.2 ± 0.2#	18.7 ± 0.1*	18.1 ± 0.1	18.6 ± 0.3
Heart (H)	17.2 ± 0.1	18.0 ± 0.1	17.7 ± 0.0	17.3 ± 0.3	18.0 ± 0.1
Signif. of difference	P<0.001	P<0.01	P<0.001	P<0.02	N.S
Brain (E)	18.8 ± 0.2	17.6 ± 0.2#	19.2 ± 0.2	18.2 ± 0.2	18.4 ± 0.3
Brain (H)	17.6 ± 0.2	18.0 ± 0.0	17.2 ± 0.2	16.7 ± 0.1	16.4 ± 0.1
Signif. of difference	P<0.001	P<0.05	P<0.001	P<0.001	P<0.001

Values are means ± S.E.M. E,euthyroid; H,hypothyroid;
n=6 , except *(n=5), #(n=7). N.S. not significant.
The mean carbon length is Σ(fatty acid mole percent x number of carbon atoms).

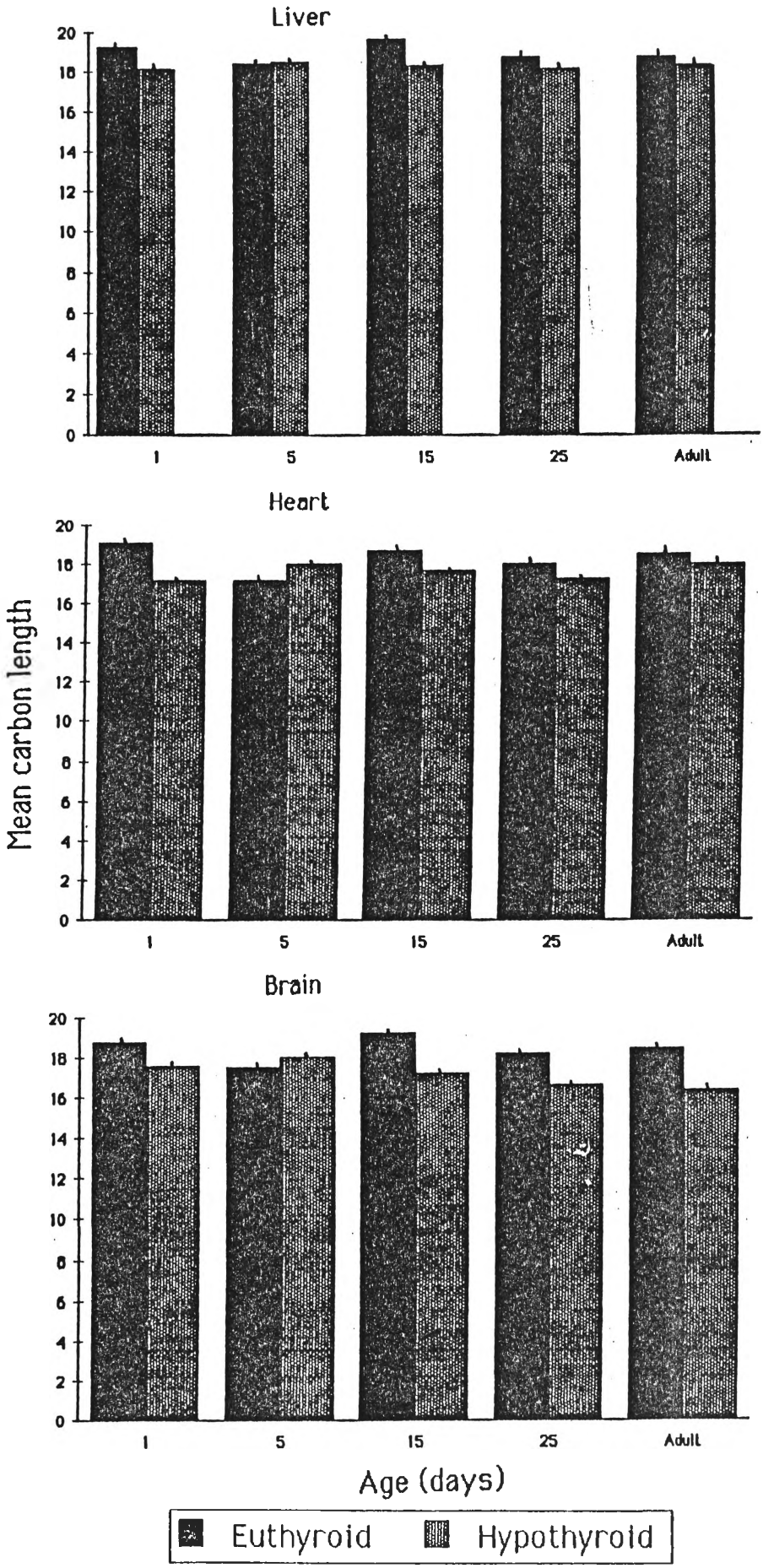

Figure 23 : The mean carbon length of phospholipid fatty acids of liver, heart and brain from euthyroid and hypothyroid rats of various ages.

4.8 Comparison of Membrane Phospholipids Fatty Acid Composition of Euthyroid Rats (25 Days) with Hypothyroid Rats (30 Days, Drinking Water Contained 0.001% PTU) at the End of Weaning.

During the course of this study, Henning published a paper which showed that weaning was delayed in hypothyroid rats (Henning 1985). It became obvious that although in 25 day old euthyroid rats weaning was complete, in 25 day old hypothyroid rats it was not complete. Henning also showed that much lower levels of PTU (0.001%) in drinking water than the 0.05% used in the present study and other developmental studies in the rat (Agostino and Henning, 1982; Wysocki and Segal, 1972; Blake and Henning, 1983) were capable of causing physiological hypothyroidism without debilitating effects of the high doses of PTU. They showed that in these hypothyroid rats weaning initiated on day 22 and was complete by day 29. For this reason I maintained a litter on 0.001% PTU and sacrificed them on day 30. All parameters which had been measured previously were also measured - this group and are compared with the values for the 25 day old euthyroid rats in Tables 17, 18 and Figure 23. The

TABLE 17 : Comparison Of euthyroid rats (25 days) with hypothyroid rats (30 days, drinking water contained 0.001% PTU) at the end of weaning.

Parameter	Organ	Euthyroid 25 days	Hypothyroid 30 days	Significance of difference
Plasma thyroxine(T_4)(nmol/lit)		107 ± 7	14.3 ± 3.5	P<0.001
Plasma triiodothyronine(T_3)(nmol/lit)		1.20 ± 0.12	0.87 ± 0.0	P<0.05
Body weight (g)		52.7 ± 2.5	72.1 ± 1.5	P<0.001
Organ weight	Liver	2.12 ± 0.10	3.39 ± 0.08	P<0.001
	Heart	0.24 ± 0.01	0.32 ± 0.01	P<0.001
	Brain	1.72 ± 0.06	1.48 ± 0.02	P<0.001
Phospholipid (mg/g tissue)	Liver	0.79 ± 0.02	0.77 ± 0.08	N.S
	Heart	0.74 ± 0.06	0.82 ± 0.04	N.S
	Brain	1.23 ± 0.08	1.18 ± 0.06	N.S
Percent Unsaturation	Liver	73.3 ± 1.3	55.3 ±0.3	P<0.001
	Heart	70.9 ± 1.7	56.0 ± 0.6	P<0.001
	Brain	70.0 ± 2.5	49.0 ±0.3	P<0.001
Unsaturation Index	Liver	250 ± 9	185 ± 3	P<0.001
	Heart	225 ± 11	175 ± 2	P<0.001
	Brain	259 ± 18	158 ± 1	P<0.001
20:4/18:2	Liver	2.41 ± 0.15	2.56 ± 0.08	N.S
	Heart	1.41 ± 0.29	1.62 ±0.08	N.S
	Brain	6.97 ± 0.64	8.20 ±0.20	N.S
Mean carbon length	Liver	18.8 ± 0.1	18.3 ± 0.1	P<0.001
	Heart	18.1 ± 0.1	17.9 ± 0.0	N.S
	Brain	18.2 ± 0.2	16.9 ± 0.1	P<0.001

Values are mean ± S.E.M (n=6).

Table 18 : Liver, heart and brain phospholipid fatty acid composition from hypothyroid rats of 30 days.

Fatty acid	Organ		
	Liver	Heart	Brain
12 : 0	0.11 ± 0.37	0.37 ± 0.02	0.07± 0.03
14 : 0	0.34 ± 0.03	0.34 ± 0.03	0.26 ± 0.02
15 : 0	0.20 ± 0.04	0.19 ± 0.15	-
X_1	0.16 ± 0.03	1.53 ± 0.02	2.62 ± 0.03
16 : 0	19.19 ± 0.58	14.62 ± 0.39	21.88 ± 0.5
16 : 1	0.70 ± 0.08	0.30 ± 0.07	0.65 ± 0.07
17 : 0	0.87 ± 0.03	0.69 ± 0.01	0.21 ± 0.04
X_2	0.15 ± 0.04	0.88 ± 0.08	3.62 ± 0.11
X_3	-	0.51 ± 0.02	1.30 ± 0.11
X_4	-	-	0.78 ± 0.07
18 : 0	23.20 ± 0.42	24.24 ± 0.27	19.3 ± 0.14
18 : 1^{δ}	6.18 ± 0.17	6.66 ± 0.30	15.03 ± 0.4
18 : 1^{Δ}	1.80 ± 0.07	3.59 ± 0.09	3.14 ± 0.09
18 : 2	10.14 ± 0.22	13.35 ± 0.72	1.36 ± 0.04
20 : 2	0.24 ± 0.82	0.22 ± 0.69	0.05 ± 0.05
20 :3	0.77 ± 0.40	0.29 ± 0.09	0.36 ± 0.03
20 : 4	25.89 ± 0.38	21.39 ± 0.50	11.09±0.27
22 : 3	1.11 ± 0.07	1.26 ± 0.05	1.69 ± 0.07
22 : 4	0.78 ± 0.06	1.93 ± 0.07	3.71 ± 0.05
22 : 5	1.96 ± 0.08	2.56 ± 0.14	0.15 ± 0.07
22 : 6	5.31 ± 0.27	4.41 ± 0.16	11.51 ± 0.1

Data are given as mole percent and values shown are the mean ± S.E.M
The fatty acids are denoted bt the number of carbon atoms in the chain followed by the number of double bonds.
$(18:1)^{\delta}$,Oleic acid; $(18:1)^{\Delta}$ Cis Vacenic acid.
X_1, X_2, X_3, X_4 are unidentified compounds.

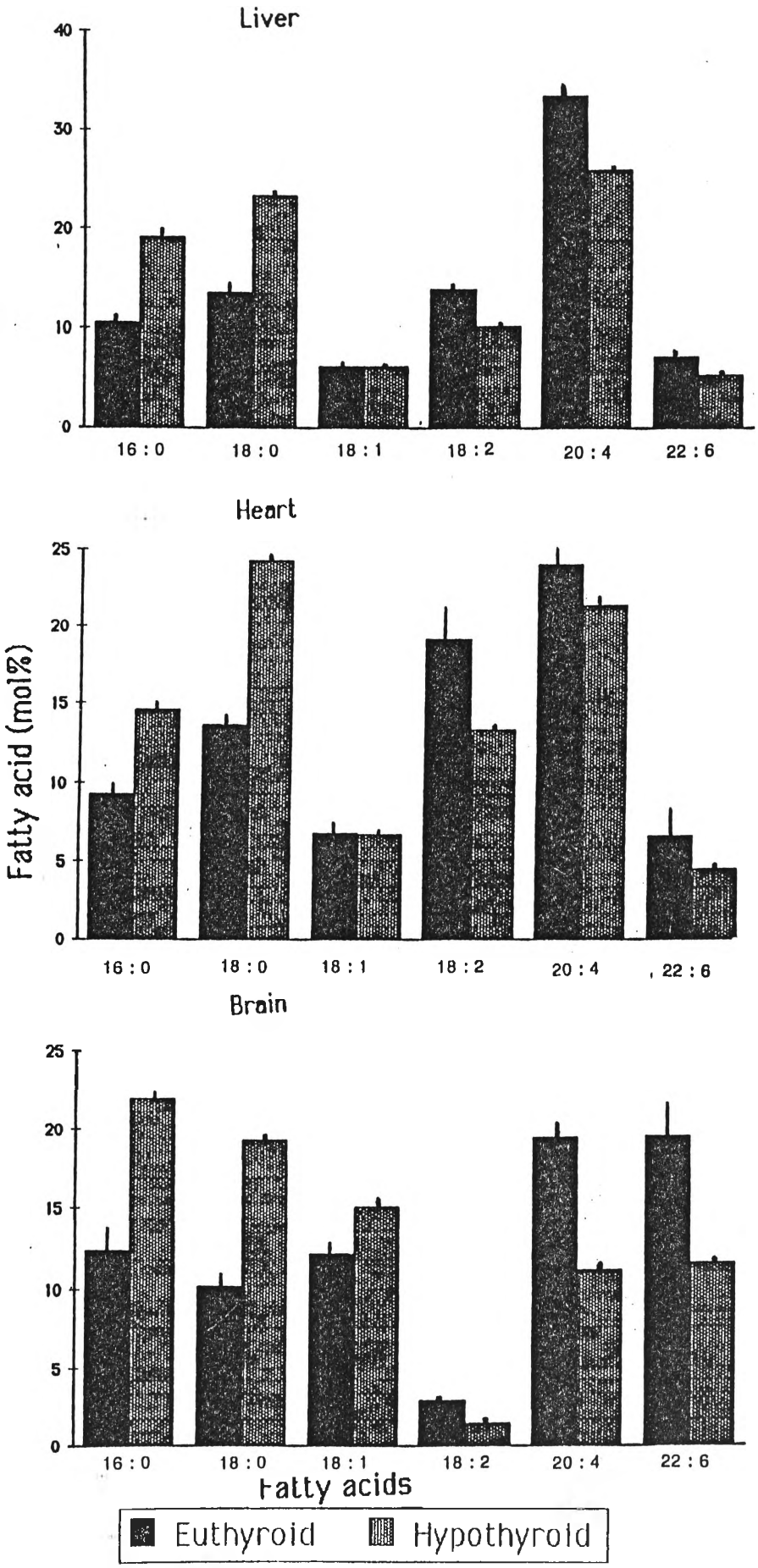

Figure 24 : Comparison of post-weaning fatty acid composition of liver, heart and brain phospholipids from euthyroid and hypothyroid rats.

result shows that plasma thyroxine and triiodothyronine concentrations are lower but body weight, organ weight, phospholipid contents are ~~the same~~ not reduced in 30 days hypothyroid rats as compared to 25 days euthyroid rats. An increase in proportion of 16 : 0, 18 : 0 and a decrease in proportion of 20 : 4, 22 : 3, 22 : 4, 22 : 6 causes lower values of percent unsaturation and unsaturation index.

CHAPTER 5

DISCUSSION

Previous studies have shown that membrane fatty acids change during development in mammals (Doviasoba et al. 1964; Kutchai et al. 1978; Pollak et al. 1982; Rovinski and Hosein,1983). Other studies have shown that thyroid activity also changes during the development of mammals (Walker et al.1982). Since thyroid hormones are known to cause changes in the membrane fatty acids of adult mammals (Patton and Platner, 1970; Hulbert et al. 1976; Steffen and Platner, 1976; Chen and Hoch, 1977; Chen and Hoch, 1976; Shaw and Hoch, 1976; Hoch et al. 1976; Hoch et al. 1981; Hoch, 1982), It may be that the developmental changes in membrane fatty acids of mammals are due to developmental changes in the thyroid activity of these young mammals. In the present study it has been found that modulation of thyroid status affects almost all of the parameters measured; i.e. plasma T_4 and T_3 concentrations, phospholipid fatty acid composition of liver, heart and brain and growth (only at 0.05% PTU induced hypothyroidism but not at 0.001% PTU induced hypothyroidism)

The only parameter that did not change was phospholipid content of these three tissues. The observed effects of thyroid status on membrane fatty acids are due to the use of PTU for chemical thyroidectomy which has been shown to reduce strongly many enzyme activities of fatty acid synthesis, i.e. acetyl Co A carboxylase, fatty acid synthetase, microsomal chain elongation and desaturation reactions. These effects were evident after three days of drug administration (Landriscina et al. 1976) and the enzyme activities did not diminish further following longer periods of PTU use.

In the present study I have not isolated individual membranes but have rather extracted total tissue phospholipids which consist of the sum total of phospholipids from all cellular membranes (i.e. plasma membrane, endoplasmic reticulum, mitochondrial membranes, nuclear envelope and lysosomal membranes). Any changes observed in such a general membrane lipid fraction need not mean every cellular membrane would show such changes or that changes are the same for each such subcellular membrane component. If only some of the subcellular membranes were affected by manipulation of thyroid and others were not then those that do

change would change their composition greatly. However, most evidence suggests that, at least in adults, that most subcellular membranes are affected by changes in thyroid activity. Nuclear, mitochondrial and microsomal membranes have all been shown to be affected by thyroid status (Patton and Platner, 1970; Hulbert et al. 1976; Steffen and Platner, 1976; Chen and Hoch, 1977; Chen and Hoch, 1976; Shaw and Hoch, 1976; Hoch et al. 1976; Hoch et al. 1981; Hoch, 1982). As shown in table 1, in the literature review (page 32-33), absence of thyroid hormone decreases unsaturation index in a variety of membranes and in some cases also decreases percent unsaturation. The decrease in unsaturation index is the most consistent of the changes due to hypothyroidism whilst percent unsaturation shows both decreases and increases in hypothyroidism. Such changes could be due to either decreased synthesis of polyunsaturated fatty acids or the possible action of thyroid hormone as an antioxidant. Polyunsaturated fatty acids have a tendency to oxidise very easily both "in vivo" and "in vitro" and antioxidants prevent this occurence. It has been shown that thyroxine can function as an antioxidant in mitochondria (Wynn, 1968 a,b) as well as in microsomal

preparations (Cash et al. 1966) preventing the peroxidation of phospholipid fatty acids in these organelles. In the present study thyroxine concentrations were lowest on day 5 and highest on day 15 in euthyroid rats (Table 2). This high concentration of thyroid hormone could reduce any peroxidation of phospholipid unsaturated fatty acids and thus cause the high unsaturation index found in the tissues of15 day old suckling rats (Table 14), conversely the low concentration of thyroxine in 5 day old suckling rats may be related to the low unsaturation index of the tissue phospholipid fatty acids at this age by allowing greater peroxidation of the polyunsaturated fatty acids.

One of the major findings of this study is that shortly after birth the phospholipid fatty acids of euthyroid rats became similar to those of the hypothyroid group. This implies that the period following birth is a period of hypothyroidism in neonatal rats. On day 1 the thyroxine concentration is similar to the adult level and there is a big difference between the euthyroid and the hypothyroid rats. At birth either the fetal thyroid is active and secreting thyroxine (Hall and Kaan, 1942) or maternal thyroxine cross the placenta (Grumbrecht and

Loeser, 1938; Knobil,1958) and appear in the fetal circulation. Either of these alterations may cause the high T_4 level observed on day 1. It is of interest that this situation applies only to thyroxine as no triiodothyronine was detected in the plasma of 1 day old rats.

Regulation of thyroid function in suckling rats and the presence of thyroxine in milk is still controversial. Strbak et al. (1974) showed the presence of thyroxine in rat's milk, the concentration of T_4 in the milk was the same as that in the plasma. Later, Glasscock and Nicoll (1984) indicated that significant passage of T_4 from mother to pup via milk does not occur in the rat. The present study supports Glasscock and Nicoll's (1984) results in that during 0-5 day period the plasma level of T_4 found in the young rat falls and presumably this indicates that the young is not receiving enough T_4 from its mother to maintain its T_4 level. This also indicates that during this period the young rat's ability to synthesize its own thyroid hormone may not also be great enough to maintain plasma levels.

Although the tissue phospholipid fatty acids show a similarity between the hypothyroid and the euthyroid group at day 5, there are still significantly greater concentrations of both thyroid hormones in the euthyroid group than in the hypothyroid rats. The reason for this anomaly is not currently explicable but it may mean that the tissues of the rat are relatively unresponsive to thyroid hormones at this age.

During weaning, which is initiated at approximately day 15 and ends at approximately 25 days in the euthyroid rat, the plasma thyroxine concentration decreases as does both the percent unsaturation and unsaturation index of fatty acids of liver and brain phospholipids. However, this is not true for the heart, which during the weaning period showed no change in the unsaturation index but a slight increase in percent unsaturation. The reason why the heart should change differently to liver and brain is unknown. Some of the changes associated with weaning may be due to the change in diet associated with weaning rather than changes in thyroid gland activity during this period. It is thus of interest that the hypothyroid group showed a similar reduction in percent unsaturation and unsaturation index over the period of day 15

to 25, to the reduction observed in the euthyroid group. This implies that the changes may be dietary rather than thyroidal in origin.

In 1 day old rats there is no measurable T_3 in either the euthyroid or the hypothyroid group but there is a big difference in T_4 concentration between these same groups. This, together with differences in the membrane fatty acids of these groups, implies that T_4 is responsible for these membrane fatty acid differences. Another interesting feature is that the plasma T_4 concentration is highest on day 15 (the initiation of weaning) whilst T_3 concentration is highest on day 25 (at the end of weaning).

During the course of this study, Henning (1985) published a paper in which she showed that the end of weaning was completed on day 30 in hypothyroid rats rather than at day 25. She also showed that hypothyroidism could be induced with a lower concentraation of PTU (0.001%). This treatment gave a low plasma concentration of T_4 and T_3. I have confirmed her results and although there was no effect of this level of

hypothyroidism on body weight or tissue weight there was a large effect of this low dose of PTU on the percent unsaturaion and unsaturation index of phospholipid fatty acids. The effects were similar to those observed in the 25 day old group where 0.05% PTU was used to induce hypothyroidism. Therefore, one can say that very low concentration of PTU significantly affects membrane fatty acid composition but seems to have no effect on the growth rate both of the whole body and the tissues.

In hypothyroid rats, the liver, heart and brain show an increased content of 16 : 0, 18 : 0 fatty acids and a decreased content of 20 : 4, 22 : 3, 22 : 4, 22 : 6 fatty acids at all ages studied. This result in the unsaturation index being low when compared to euthyroid rats (except 5 day old rats in liver, heart and brain and 15 day old rat in heart). This result is in agreement with that of Hoch et al. (1981) who injected 1-^{14}C linolenate (18 : 2) into both euthyroid and hypothyroid rats. They found a defective conversion of 18 : 2 to 20 : 4 in the hypothyroid rats while the euthyroid rats converted 18 : 2 almost exclusively to 20 : 4. The hypothyroid animals transferred more ^{14}C 18 : 2 label into 16 : 0 and 18 : 0 than

into 20 : 4. These authors also suggested that diminished incorporation of labelled linoleate into lipids (liver) may be connected with decreased liver coenzyme concentrations (Tabachnick and Bonny Castle, 1954) and depressed fatty acid synthesis during hypothyroidism (Dayton et al. 1960).

In this thesis, I have examined changes in the structural components of membranes, these may and probably do have functional implications. For instance, it has been indicated that permeability and enzyme activities of membranes are affected by the fatty acid composition of these membranes. Permeability studies with glycol, glycerol, erythrol (Degier et al. 1968) and glucose (Demel et al. 1968) show that this noncarrier mediated transport increases with the increasing unsaturation of phospholipid fatty acids. Permeability and enzyme activities of some membranes are also affected by thyroid hormones (Haber and Loeb, 1984 ; Ismail Beigi and Edelman 1970). Thyroid hormones have effects at the level of the cell membrane serving to increase the uptake of some amino acids and carbohydrates (Bermal and De Groot, 1980). The known high binding affinity of thyroxine for phospholipids makes the concept of a membrane site of action

attractive (Hiller, 1970; Hulbert, 1978). Some studies have suggested an involvement of thyroid hormones in the Na^{+} K^{+} ATPase and Ca^{++} Mg^{++} ATPase system (Ismail Beigi and Edelman, 1970; Galo et al. 1981). one of these studies showed that the thyroid hormone effects varied according to the fatty acid composition of the membrane lipids, which was in turn, affected by the composition of the fat supplemented diets (Galo et al. 1981). Further studies of development would be worthwhile in order to determine exactly what functional aspects are affected.

SUMMARY

The role of the thyroid in developmental changes in membrane fatty acids was examined in the rat. Three tissues were studied in euthyroid and hypothyroid rats, the tissues were liver, heart and brain. Hypothyroidism was acheived by providing pregnant and young rats with drinking water containing 0.05% PTU. Both euthyroid and hypothyroid rats were killed at 1, 5, 15 and 25 days of age as well as adult rats. The following parameters were measured in both groups ; plasma T_4 and T_3 concentrations, body weight, tissue weights, phospholipid content of tissues, fatty acid composition of phospholipids isolated from these tissues.

Plasma concentrations of T_4 and T_3 showed that the PTU treated were indeed hypothyroid. Plasma T_4 and T_3 concentrations changed with age in euthyroid rats with peaks at 15 days and 25 days respectively. Upto day 15 both in euthyroid and hypothyroid rats showed the same growth rate. Euthyroid rats grew at a much faster rate than the hypothyroid rats. Organ weights were also reduced in hypothyroid rats compared to euthyroid rats. Much reduced organ weights were

found in 25 day old and adult hypothyroid rats compared to the euthyroid rats.

The composition of fatty acids in the phospholipids changed with age and differed between organs in euthyroid rats. The phospholipids' fatty acid composition of liver, heart and brain differed with age more markedly in euthyroid rats and less markedly in hypothyroid rats in all organs studied and there was a large difference between the hypothyroid and euthyroid groups.

The unsaturation index of the phospholipid fatty acids was determined. In the hypothyroid rats, unsaturation index was lower than the euthyroid rats in all organs and at all ages (except 5 day rats) studied. This was due predominantly to decreased content of 20 : 4 (arachidonic acid) and other polyunsaturated fatty acids i.e. 22 : 3 (docosatrienoic acid), 22 : 4 (docosatetraenoic acid) and 22 : 6 (docosahexaenoic acid). This possibly resulted in a changed in membrane fluidity of these membranes.

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