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ASPECTS OF LIPID METABOLISM IN THE SUBCELLULAR FRACTIONS OF THE EMBRYO AND NEONATAL CHICK

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by

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To my parents for their endless support and

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CONTENTS

VOLUME 1

ABBREVIATIONS

et al.	.....	And others
HPLC	.....	High Performance Liquid Chromatography
COMA	.....	Committee on Medical Aspects of Food Policy
FAWC	.....	Farm Animal Welfare Council
NACNE	.....	National Advisory Committee on Nutrional Education
MAFF	.....	Ministry of Agriculture, Fisheries and Food
IRCS	.....	International Research Communications System
FEBS	.....	Federation of European Biochemical Societies
HMSO	.....	Her Majesty's Stationary Office
Inst. Natl.	.....	National Institute
plc.	.....	Public limited company
Ltd.	.....	Limited
Inc.	.....	Incorporate
®	.....	Registered
SE	.....	Standard error of the Mean
d	.....	Days
DO	.....	Day-old
%	.....	Percentage
°C	.....	Degree centrigrade
DB	.....	Dry bulb temperature
WB	.....	Wet bulb temperature
Å	.....	Angstroms
v/v	.....	Volume for volume
w/v	.....	Weight for volume
rpm	.....	Revs per minute
g	.....	Centrifugal force (gravity)
g	.....	Gram(s)
mg	.....	Milligrams
μg	.....	Micrograms
μg/g	.....	Micrograms per gram
ml	.....	Millilitre
μl	.....	Microlitre
M	.....	Molar
mM	.....	Millimolar
μM	.....	Micromolar
pM	.....	Picamolar
cm	.....	Centimetres
μm	.....	Micrometre
nm	.....	Nanometres
psi	.....	Pounds (in weight) per square inch

α	.....	Alpha
ß	.....	Beta
ɣ	.....	Gamma
σ/Δ	.....	Delta
Δ4-	.....	C4-C5 double bond
Δ5-	.....	C5-C6 double bond
Δ6-	.....	C6-C7 double bond
Δ9-	.....	C9-C10 double bond
sn-1	.....	Fatty acid esterified to the first carbon of the trihydric alcohol glycerol.
sn-2	.....	Fatty acid esterified to the second carbon of the trihydric alcohol glycerol.
trans-	.....	Fatty acid groups on adjacent sides of the C=C double bond forming an assymetrical molecule
cis-	.....	Fatty acid groups on the same side of the C=C double bond forming a symmetrical molecule.
C2	.....	Fatty acid with two carbons
C18	.....	Fatty acid with eighteen carbons
C20	.....	Fatty acid with twenty carbons
C22	.....	Fatty acid with twenty-two carbons
16:0	.....	Palmitic acid
16:1	.....	Palmitoleic acid (n-7 fatty acid)
18:0	.....	Stearic acid
18:1	.....	Oleic acid (n-9 fatty acid)
18:2	.....	Linoleic acid (n-6 fatty acid)
18:3	.....	Linolenic acid (n-3 fatty acid)
20:4	.....	Arachidonic acid (n-6 fatty acid)
22:5	.....	Docosapentaenoic acid (n-3 fatty acid)
22:6	.....	Docosahexaenoic acid (n-3 fatty acid)

In the abbreviated formula the chain length is given followed by a colon which in turn is followed by the number of double bonds in the molecule. The position of the terminal double bond is donated by *n* (the number of carbon atoms in the chain) minus the number of carbon atoms between the terminal double bond and the methyl group.

PL	.....	Phospholipid
FC	.....	Free (unesterified) cholesterol
FFA	.....	Free fatty acids
TG	.....	Triglyceride
CE	.....	Cholesterol ester
PG	.....	Partial glycerides (Mono- and Diglycerides)

E.C. Abbreviations

Phospholipase	.....	3.1.1.4
Cytochrome C oxidase	.....	1.9.3.1
Adenosine triphosphatase	.....	2.7.2.1
Glucose 6-phosphate	.....	3.1.3.9
Nicotinamide Adenine Dinucleotide (reduced) :cytochrome c oxidase	.....	1.6.99.3
Nicotinamide Adenine Dinucleotide (reduced) :cytochrome b_5 reduction complex	.....	1.6.22
Lipoprotein lipase	.....	3.1.1.34
Glutathione peroxidase	.....	1.11.1.9
Catalase	.....	1.11.1.6
Superoxide dismutase	.....	1.15.1.1
Cytochrome P-450scc	.....	1.14.15.6
ß-hydroxybutyrate	.....	1.1.99.6
Succinoxidase	.....	1.3.1.6

LIST OF FIGURES

LIST OF PLATES

CHAPTER 2

CHAPTER 4

LIST OF TABLES

SUMMARY

The parental age of the broiler bird is known to exert a significant effect on embryo hatchability and early chick survival. In order to increase chick yield to the broiler industry, there is a need to procure hatching eggs at earlier stages of the hen's reproductive life. However reduced hatchability of eggs from young parents has affected the economics of such a move and has thus led to the eggs from young parents being discarded. Results to date have shown that there are extensive disturbances to yolk lipid uptake and the subsequent assimilation of lipid by the embryonic tissues.

The present study was concerned with comparative changes in lipid and fatty acid compositions of the subcellular lipid, together with aspects of tissue lipid metabolism in embryos and neonatal chicks derived from mature and young parents.

Fertile eggs were obtained from Ross 1 broiler-breeder parent stock at twenty-three to twenty-four and then again at thirty-seven to forty weeks of age.

Embryos and chicks from each parental group were randomly selected at predetermined intervals during incubation and post-hatch development. Tissue organelles were separated by standard techniques following suitable modification. Preparations for electron microscopy were also made by standard techniques.

Lipid extraction and subsequent analyses of lipid moieties and fatty acids were performed by a range of methodologies which included differential solvent extraction, Thin Layer chromatography, Gas Liquid chromatography and High Performance Liquid chromatography.

As shown by electron microscopy, the mitochondria exhibited typical pleomorphism throughout the embryonic and post-hatch periods. Absolute amounts of lipid associated with the liver cytoplasm of embryos from mature parents increased dramatically over the last week of incubation. In embryos from young parents, the lipid accumulation was significantly less.

There was a rapid diminution in the levels of cytoplasmic lipid in the livers of chicks of mature parents following hatching. By comparison, the rate of lipid loss was less in the livers of chicks from young parents. The levels of lipid associated within the membrane organelles were, throughout the embryonic and post-hatch periods, consistently higher than within the cellular supernatant. Within the subcellular fractions from embryos of young parents, the levels of lipid within the mitochondria of the liver, heart and brain were higher compared with those from mature parents.

Phospholipids and free cholesterol were the major lipid fractions within the mitochondria and microsomes. The phospholipids of the liver mitochondria and microsomes from embryos of both parental groups, showed unique changes in their saturated to polyunsaturated fatty acid pairings during the final week of incubation, suggesting the differentiation in phospholipid turnover and associated changes in membrane organelle stability and function.

Lipid and fatty acid changes in the heart organelles were very similar to those of the liver, suggesting similar relative metabolic changes in spite of proportional differences in both lipid and fatty acid compositions. The mitochondria of the brain were

characterised by high levels of docosahexaenoic acid, whilst the levels of linoleic and linolenic acids were exceedingly low.

Cholesteryl esters, in particular cholesterol oleate, comprised by far the bulk of the supernatant fractions of the liver and heart. A marked increase in the relative concentration of cholesteryl esters was observed within the liver during the final week of incubation. By comparison, the brain supernatant displayed an exceedingly low level of cholesteryl esters throughout the embryonic and post-hatch periods. The level of cholesteryl esters and of cholesterol oleate in particular, decreased following hatching.

The triglycerides of the liver uniquely showed, during the final week of incubation, a very high concentration of docosahexaenoic acid, the level of which decreased rapidly during the early post-hatch period. In contrast, the concentration of docosahexaenoic acid within the triglycerides of the brain organelles was very low. A marked increase in the relative concentration of triglycerides occurred in both the subcellular fractions of the liver and heart following hatching, associated in particular with the rapid reduction in cholesterol ester level.

A range of significant differences in both lipid and fatty acid compositions of the liver and its organelles between progeny from mature and young parents was observed, reflecting disturbances in lipid metabolism within the embryos from young parent stock. Changes in the free cholesterol:phospholipid ratio in the liver mitochondria, in

particular, are associated with alterations in fatty acid composition, suggesting disturbances in membrane permeability and stability.

Although differences were noted in lipid and fatty acid compositions between the heart and brain organelles of progeny from mature and young parent stock, their extent appeared subordinate to those of the liver.

During embryonic development, there was an extremely high emphasis placed on the accumulation of C20 and C22 polyunsaturated fatty acids. Investigations of their protection, via alpha tocopherol, provided by the yolk, indicated that polyunsaturated fatty acid accumulation within the tissues was not coincident with the required 'build up' in alpha tocopherol concentrations. Whereas polyunsaturated fatty acid levels were maximal at day 15 of incubation, alpha tocopherol levels were maximal at day 19, commensurate with their transport, via lipid assimilation, from the yolk sac membrane. Observations in embryos from young parents, of polyunsaturated fatty acid to alpha tocopherol accumulations, suggested an enhanced risk with respect to polyunsaturated fatty acid protection.

Measurements of free cholesterol accumulation within the brain during the final week of incubation suggested that, during embryonic development, the brain was capable of regulating cholesterol supply, via yolk absorption or synthesis, according to demand. Differences observed in cholesterol and desmosterol accumulations within the brain of embryos from young parent stock, implicated disturbances in the ability to regulate cholesterol supply for brain development.

The changes in lipid and fatty acid composition in both the embryonic and post-hatch periods, within the subcellular organelles of the tissues, underline the important role of lipid metabolism in neonatal development. Observed differences between progeny from mature and young parent stock, indicate the possible involvement of alterations in fundamental aspects of lipid metabolism in ultimate chick survival and livability.

CHAPTER 1
GENERAL INTRODUCTION

The extent of the qualitative and quantitative lipid changes that take place in the organelles of animal tissues during development is a question that remains largely unresolved. In reviewing the available data, it is obvious that contradictions abound, not least because of the wide variation in conditions under which the studies have been performed. In spite of these reservations, it is apparent that changes which occur within an organelle during development can still provide an insight into the mechanistic nature of a range of metabolic features involved in the overall differentiation of the cell.

Extensive studies have been undertaken on mammalian cells in culture with respect to the lipid composition of membranes and their influence on membrane function. Information on the involvement of essential fatty acids in electrolyte and non-electrolyte transport systems has been obtained using artificial membrane systems (Chen, Lund and Richardson, 1971). Studies have also shown that the permeability properties of membranes can be markedly influenced by the fatty acid composition of the phospholipids and in particular, by their degree of unsaturation (Gier De, Mandersloot and Van Deenen, 1968; Berlin, Bhathena, Judd and Taylor, 1989); expected proportional 'increases in fluidity ' did not necessarily occur when the unsaturation of membranes was increased (Herring, Tatischeff and Weeks, 1980; Stubbs, Tsang, Belin, Smith and Johnson, 1980; McVey, Yguerabide, Hanson and Clark, 1981). Nevertheless, the findings were in general accord with the statement (Chen *et al.*, 1971) that phospholipids containing long-chain saturated fatty acids formed tightly packed bilayers with relatively low permeability, as opposed to phospholipids containing unsaturated fatty acids which produced more permeable membranes due to the reduced attractive forces between adjacent lipid molecules.

Bretscher (1972) presented evidence of an asymmetric distribution of phospholipids in red cell membranes. Such asymmetry was subsequently demonstrated within the membranes of mitochondria (Marinetti, Senior, Love and Broadhurst, 1976; Nilsson and Dallner, 1977; Crain and Marinetti, 1979; Krebs, Hauser and Carafoli, 1979; Harb, Comte and Gautheron, 1981) and microsomes (Higgins and Dawson, 1977). However, the extent of the asymmetric structures within the microsomal phospholipids reported by different laboratories are conflicting (Nilsson and Dallner, 1977; Sundler, Sarcione, Alberts and Vagelos, 1977; Bollen and Higgins, 1980).

The role of lipids in membrane structure and stability is well documented (see Lee, 1983; see Brenner, 1984; see Quinn, Joo and Vigh, 1989). The lipid bilayer of the membrane can exist in two distinct physical states, a gel phase (equivalent to a solid) in which the phospholipids are tightly packed with their fatty acyl chains fully extended and a liquid crystalline phase (equivalent to a liquid-like state) in which there is a considerable rotation about the carbon to carbon bonds of the fatty acyl chains and lateral diffusion of the phospholipids within the plane of the membrane. In the gel phase, the 'brittleness' of the membrane is such that the cell becomes osmotically fragile (see Melchior, 1982) and for adequate functioning of the membrane proteins it is necessary to maintain a fluid environment. Consequently, the phospholipids must change to ensure that lipids are in the correct liquid crystalline state at physiological temperatures. The required enthalpic change from an ordered, condensed state to a fluid, expanded state known as phase transition, is generated by rotamery of carbon to carbon bonds of hydrocarbon tails from an antiplanar (*trans*) to a cylindrical (gauche) structure (Jackson, 1976). The phase transition is usually preceded by a pre-transition stage at a lower temperature (Hinz and Sturtevant, 1972; Sackmann, Träuble, Gaila and Overath, 1973). In most cases, as in phospholipid mixtures, there is no sharp transition temperature, but rather a smooth change

between temperature limits (Thilo, Träuble and Overath, 1977; Garda and Brenner, 1982).

In addition to the length of the fatty acyl chains and the degree of unsaturation, the temperature of transition between the gel and liquid crystalline phasic states depends also on the class of phospholipid. As dictated by the presence of methyl groups, phosphatidylcholines have lower transition temperatures than phosphatidyl-ethanolamines (Vaughan and Keough, 1974). It is generally accepted that short chain fatty acyl chains result in lower transition temperatures, although phospholipids containing fatty acid chains shorter than myristic acid do not form stable bilayers (see Lee, 1985).

Changes in the composition of the phospholipid bilayer, particularly if they involve the unsaturated fatty acids with the *cis*-double bond at the centre of the chain (as in oleic acid), favour the liquid crystalline phase and are of potentially great importance to membrane function. The increase in lipid bilayer fluidity by *cis* unsaturated fatty acids has been demonstrated using electron spin resonance (Seelig and Seelig, 1977). However little work has been done to elucidate the relationships between lipid composition and permeability in biological systems since large changes in the fatty acid composition of the cell membranes are very difficult to control.

Nevertheless, studies on the effects of multiple unsaturation (Coolbear, Berde and Keough, 1983) have shown that the introduction of the first double bond favours the liquid crystalline phase; the introduction of a subsequent double bond, in either the *cis* or *trans*- position, has less of an effect, whilst further bonds in fact tend to cause a slight increase in transition temperature (Coolbear *et al.*, 1983). A *trans*-double bond however, evokes less of a decrease in transition temperature (Sackmann *et al.*, 1973) than a *cis*-bond. The presence of a *cis*-double bond within the membrane

structure prevents the fatty acyl chain from assuming a completely extended configuration but, being fixed structures, they introduce rigid elements in the otherwise flexible hydrocarbon chain (Seelig and Seelig, 1977; Thulborn, Treloar and Sawyer, 1978). The number of the rigid elements will clearly increase with the degree of unsaturation of the fatty acid.

Lipid structural order in membranes at physiological temperatures depends also on the free cholesterol and sphingomyelin content as well as the degree of saturation of the phospholipid acyl chain (Kawato, Kinosito and Ikegami; 1978; Blitterswijk Van, Van Hoeven and Van der Meer, 1981). The role of cholesterol in the cell membrane is well documented (see Barenholz, 1984; see Yeagle, 1985a,b) but emphasis has been placed on the dual effect this sterol has on the fluidity of the bilayer (Ladbrooke, Williams and Chapman, 1968; Lippert and Peticolas, 1971; Oldfield and Chapman, 1971). In the liquid-crystalline phase, cholesterol has a condensing (ordering) effect on the packing of the phospholipids (Kawato *et al.*, 1978) leading in general to a reduction in membrane fluidity that is dependent on the molecular species of the lipids involved (Demel, Jansen, Van Dijck and Van Deenen, 1977) and their degree of fatty acyl unsaturation (Demel, Geurts van Kessel and Van Deenen, 1972), whilst in the gel phase, the ordered array of lipid acyl chains is fluidised in the presence of cholesterol (Kawato *et al.*, 1978). Sphingomyelin not only exerts a 'lipid ordering' effect (a process known as rigidifying), but has been shown to interact preferentially with cholesterol (Demel *et al.*, 1977) and may enhance lipid phase separation into membrane domains of varying order (Blitterswijk Van *et al.*, 1981; Blitterswijk Van, De Veer, Krol and Emmelot, 1982).

Modifications to the ratio of free cholesterol: phospholipid in the membrane (Vanderkooi, Fischoff, Chance and Cooper, 1974; Cooper, Leslie, Shinitzky and Shattil, 1978), or to the fatty acid composition, can be extensive enough to promote an alteration in a range of functions. These include: membrane fluidity (King and Spector, 1978; Burns, Luttenegger, Dudley, Buettner and Spector, 1979; Berlin *et al.*, 1989); membrane stability against certain conditions, for example, haemolytic stress (Noble, Shand, Drummond and Moore, 1977; Shand and Noble, 1981a); many cellular functions including carrier-mediated transport (Kaduce, Awad, Fontelle and Spector, 1977; Burns *et al.*, 1979; Yorek, Bohnker, Dudley and Spector 1984); the properties of certain membrane bound enzymes (Engelhard, Esko, Storm and Glaser, 1976; Sinensky, Minneman and Molinoff, 1979; Pagano and Longmuir, 1985); receptor binding (Axelrod, Wight, Webb and Horwitz, 1978; Ho and Cox, 1982; Bar, Dolash, Spector, Kaduce and Figard 1984; Berlin *et al.*, 1989); phagocytosis (Lokesh and Wrann, 1984); endocytosis (Mahoney, Hamill, Scott and Cohn, 1977); depolarisation-dependent exocytosis (Williams and McGee Jr, 1982); chemotherapeutic cytotoxicity (Yoo, Chiu, Spector, Whiteaker, Denning and Lee, 1980; Yoo, Kuo, Spector, Denning, Floyd, Whiteaker, Kim, Kim, Abbas and Budd, 1982); prostaglandin production (Denning, Figard and Spector, 1982; Kaduce, Spector and Bar, 1982) and cell growth (Doi, Doi, Schroeder, Alberts and Vagelos, 1978; Spector, Kiser, Denning, Koh and Debault, 1979).

However alterations in the free cholesterol:phospholipid ratio can also lead to compensatory adjustments that include for example, an increase in membrane unsaturated fatty acid levels (Baldassare and Silbert, 1979; Rintoul, Chow and Silbert, 1979). Such compensatory changes decrease the temperature for the phase transition and thereby preserve the fluid state of the plasma membrane at physiological temperatures (Untracht and Shipley, 1977; Baldassare and Silbert, 1979; Rintoul *et al.*, 1979).

Significant differences in membrane lipid composition and viscosity have been produced by dietary means (Neudoerffer and Lea, 1967; Yao, Holman, Lubozynski and Dyck, 1980; Innis and Clandinin, 1981; Tahin, Blum and Carafoli, 1981). Such changes may be affected both directly and indirectly. Direct changes may simply arise from dietary substitution of fatty acids with possible consequential effects on lipid compositions and structures. Indirect changes are more difficult to delineate.

The membrane lipids of subcellular organelles, in particular the phospholipids, are by their very nature susceptible to oxidative degeneration (Combs, Noguchi and Scott, 1975) and since they form non-aqueous environments within the cells, a fat soluble antioxidant may be required to maintain membrane stability. Furthermore, biological membranes contain active catalysts such as haemoproteins and non-haeme iron, copper and manganese complexes, which enhance lipoperoxidation (Toyoda, Terao and Matsushita, 1982) and oxidation of even a small portion of the unsaturated fatty acids in the phospholipid bilayer may induce considerable and often irreversible damage to the cells of many types of tissues (Bulkley, 1983; Marx, 1987).

In addition, cellular damage that may arise via the deterioration of barrier and matrix function of the lipid bilayer and enzyme inactivation, can also be caused by the products of lipid oxidation interacting with the biological material contained in the cells themselves. Changes in the diet, such as an increase in polyunsaturated fatty acids without commensurate protection from appropriate intakes of tocopherols and other antioxidants, may in turn lead to or accelerate *in vivo* oxidation causing potential damage (Hoffman, 1962; Peers, Coxon and Chan, 1981), especially under unfavourable conditions such as large intakes of antioxidant substrate and high temperature (Cillard, Cillard and Cormier, 1980). Toxic metabolites such as hydrogen peroxide, superoxide, hydroxyradical and singlet oxygen, can also initiate

cellular damage to other pathways causing molecular disruption of nucleic acids and proteins. The polymeric products formed via free radicals may lead to the formation of intermolecular cross linkages creating rigid sites within the membranes (see Mead and Alfin-Slater, 1966). As an alternative mechanism to that provided by a synthetic antioxidant, alpha (α) or gamma (γ) tocopherol can offer efficient protection against autoxidation, although α-tocopherol is more effective than γ-tocopherol (Wu, Stein and Mead, 1979). In the case of free cholesterol, it has been shown that in responses to ultra violet radiation, cholesterol epoxide, a known carcinogen, may be formed in the skin (Black and Douglas, 1972).

Thus, although both polyunsaturated fatty acids and cholesterol are considered to be essential for the maintenance of normal biological function, their susceptibility to peroxidation may, under certain circumstances, put their beneficial aspects at risk. The effects of lipid modification on cellular function are consequentially very complex and it is difficult to make any generalisation or to predict how a particular system will respond to a certain type of lipid modification. Furthermore, as noted previously, a number of inconsistencies in the observations so far make it difficult to draw any conclusions as to how a system will react to any particular type of lipid modification. Nevertheless, it would seem that many of the functional responses observed are caused directly by changes in the structure of the membrane lipids which affect either bulk fluidity or specific areas noted to be rich in lipids, or a combination of both (Karnousky, Kleinfield, Hoover and Klausner, 1982). It has also been suggested (see Spector and Yoreck, 1985) that the lateral mobility of the proteins, or their interactions with other membrane components, may also be affected as a result of structural changes in the conformation or binding abilities of the proteins to the lipid moieties.

Clearly, membrane lipids, in particular the phospholipids, need to be considered in relation to their overall metabolism and not just in terms of an isolated role within the membrane. The phospholipids of the plasma membrane for example, are important in cell stimulation when phosphatidylethanolamine is transmethylated to phosphatidylcholine (Hirata and Axelrod, 1980) and turnover of phosphatidylinositol is increased (Michell, Kirk, Jones, Downes and Creba, 1981).

CHAPTER 2
LITERATURE REVIEW

1 The nature and classification of lipids.

The term 'Lipid' is used to describe a wide range of naturally occurring products and their derivatives which are readily soluble in organic solvents but insoluble in water. Nowadays, the term is restricted to fatty acids and their derivatives or metabolites (see Christie, 1982). The principal lipid classes consist of fatty acid (long-chain aliphatic monocarboxylic acid) moieties linked by an ester bond to an alcohol, principally the trihydric alcohol glycerol, or by amide bonds to long chain bases. In addition, they may contain other moieties for example, phosphoric acid, organic bases or sugars. Lipids can be divided into two main groups; simple lipids which, when hydrolysed, give one or two different types of product per molecule (for example, tri-, di- or monoglycerides, esterified and unesterified (free) cholesterol, free fatty acids) and complex lipids, which contain three or more hydrolysis products per molecule (for example, phospholipids, sphingolipids). The terms 'neutral' and 'polar' respectively are used more often to define these classes (see Christie, 1982).

Since lipids are apolar, their dissolution in the aqueous environment of the plasma and elsewhere in the body is achieved through the formation of lipid-protein complexes known as lipoproteins. In this form, the naturally hydrophobic lipids are able to be transported from one body site to another. The nature of the lipid-protein binding is poorly understood but nevertheless, methods have been devised for their division into major discrete classes of families which possess both metabolic and compositional distinctions (see Christie, 1982). The common division of the lipoproteins into high density, low density and very low density fractions is indicative of the methods of their isolation based on flotation by ultracentrifugation and electrophoresis (see Perkins, 1975; see Kuksis, 1978; see Christie, 1982; see Christie and Noble, 1984).

2 The yolk lipids.

2.1 Deposition of the yolk lipid.

No dissertation of the biochemistry and nutrition of the avian embryo is possible without a consideration of the starting point, namely the yolk. Extensive literature exists that describes the yolk and its various components (see Romanoff and Romanoff, 1949; see Gilbert, 1971; see Griminger, 1976). With the onset of laying, the normal lipid patterns in the hen undergo considerable changes (Yu, Campbell and Marquardt, 1976; Chapman, 1980). In the liver there is a dramatic increase in the overall concentration of lipids which is accounted for, in particular, by an increase in the level of triglycerides (Lorenz, Chaikoff and Entenman, 1938; Husbands and Brown, 1965). Similar increases in the concentrations of phospholipids and free cholesterol have not been observed, although kinetic studies have suggested that their turnover within the liver is significantly increased (Taurog, Lorenz, Entenmann and Chaikoff, 1944). The changes in plasma lipid concentration which precede egg laying are associated with a dramatic rise in the concentration of the triglyceride-rich very low density lipoprotein fraction, as a result of increased synthesis within the liver (Kudzma, St. Claire, DeLallo and Friedberg, 1975) and reduced clearance from the plasma (Bacon, Brown and Musser, 1973). There is little, if any change in the concentrations of the low and high density lipoprotein fractions. These changes in gross lipid levels are accompanied by alterations in fatty acid composition (Chung, Ning and Tsao, 1966; Balnave, 1969), subsequently reflected in the yolk lipid fatty acids.

2.2 Separation of the yolk components.

The yolk contents show considerable inhomogeneity (see Gilbert, 1971; see Griminger, 1976); a division of avian yolk into white and yellow components has been recognised for a long time. The white yolk, which constitutes only about 1 to 2 per cent, is associated with the germinal disc and is thus deposited during early ovum maturation. The majority of the yolk mass is therefore associated with the yellow yolk and exists in the form of large floating spheres (25 to 150μm diameter) emulsified in a continuous aqueous-protein phase. Its deposition during the later stages of ovum formation determines that it contains the majority of the yolk lipid, whilst much smaller granules (up to 2μm diameter) exist within the spheres and aqueous phase (Bellairs, 1961; see Gilbert, 1971). The series of concentric layers of the yellow yolk is merely representations of the cyclic rates of lipid deposition.

Almost all the yolk lipid is present as lipoprotein complexes and has an overall lipid: protein ratio of about 2:1 (see Noble, 1987a). Low speed centrifugation produces a sediment of the granular fractions which accounts for about 25 per cent of the total yolk solid and 7 per cent of the total yolk lipid (see Cook, 1968; Gornall and Kuksis, 1973). Further separation of the granular fraction using electrophoresis or ion exchange chromatography yields two high density lipoprotein fractions (the α and ß lipovitellins) and a small amount of a low density triglyceride-rich lipoprotein. Prolonged centrifugation at a much higher speed yields the vast bulk of the yolk lipid (greater than 90 per cent) which is associated with the low density triglyceride-rich fraction found within the large spheres (Gornall and Kuksis, 1973). This fraction has been found to have a very similar structure to that of the mammalian very low density lipoprotein (Gornall and Kuksis, 1973) and can be further subdivided by centrifugation or gel filtration (see Griffen, Perry and Gilbert, 1984). A very small amount of residual lipid may be recovered from the aqueous infranatant.

3 Lipid metabolism in the chick embryo.

The average 60g chicken's egg contains 6g of lipid which is a major nutritional component for the developing embryo, supplying both energy and essential nutrients. During the development of the chick embryo, more than 90 per cent of the total energy requirements is derived from fatty acid oxidation (see Romanoff, 1967; see Freeman and Vince, 1974). The oxidative requirements and mechanisms of the process have been shown to be similar to those for fatty acid oxidation in mammalian tissues (Koerker and Fritz, 1970; Pugh and Sidbury, Jr., 1971) and are identifiable from about the eighth day of incubation (Pugh and Sidbury, Jr., 1971). The extensive availability of lipid substrates within the egg eliminates the need for lipogenesis which thus, although detectable, remains very low during embryonic development (Goodridge, 1968, 1973a, b; Joshi and Sidbury, Jr., 1976).

The process of assimilation of the large concentration of yolk lipid during the last seven days of incubation (Plate 1a b) is associated with some notable changes in tissue lipid and fatty acid compositions (Noble and Moore, 1964, 1967a,b,c). Moreover, recent investigations into the concentration changes of some of the major lipid classes and their fatty acid compositions within the yolk complex and embryo have indicated that enzyme activity, cellular morphology and ultrastructure of the yolk sac membrane and tissues are directly linked to the changing extent and rate of yolk lipid assimilation by the embryo and its subsequent catabolism (Noble and Shand, 1985; Noble and Yafei, 1988; Yafei and Noble, 1990). Absorption of the yolk lipid forms an indispensable part in the successful development and emergence of the chick; the triglycerides act as the most important source of energy for the embryo and the phospholipids provide essential constituents for tissue development and function. Changes in the normal pattern of yolk lipid mobilisation, assimilation and utilisation,

Plate 1

Chick embryos at days 13 and 19 of incubation (1a and 1b respectively) showing the rapid growth of the embryo and utilisation of the yolk during this period.

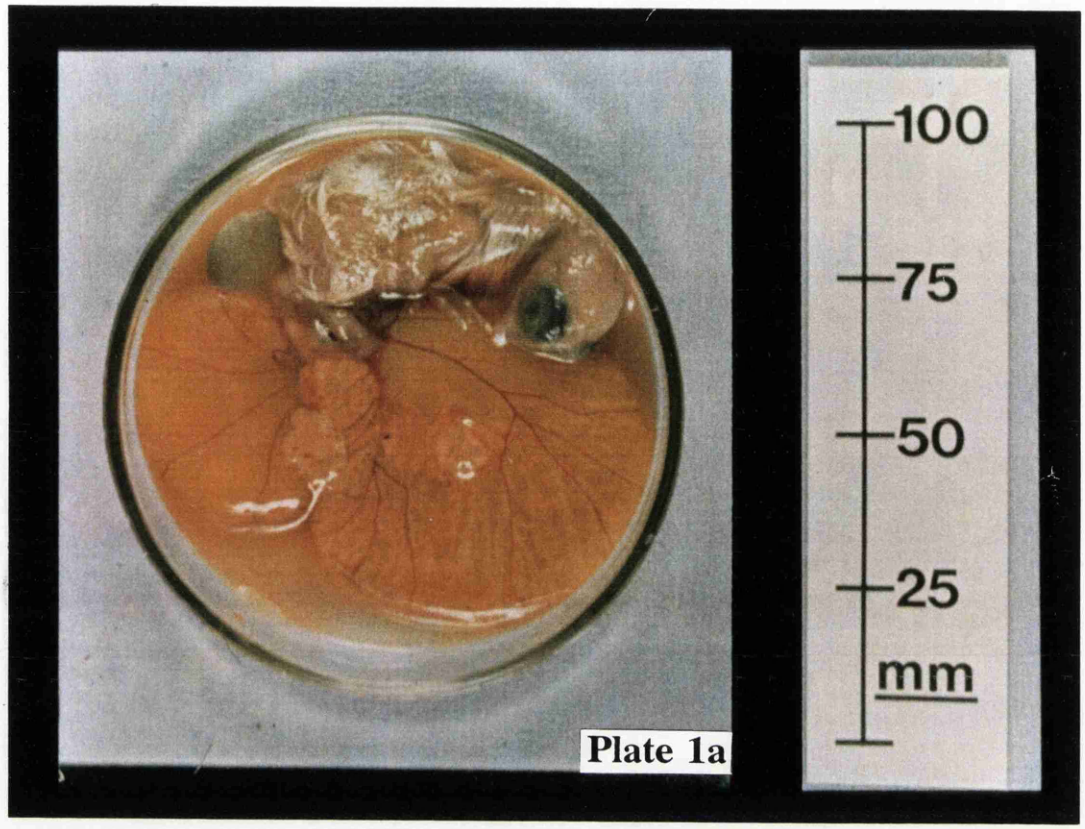


Plate 1a

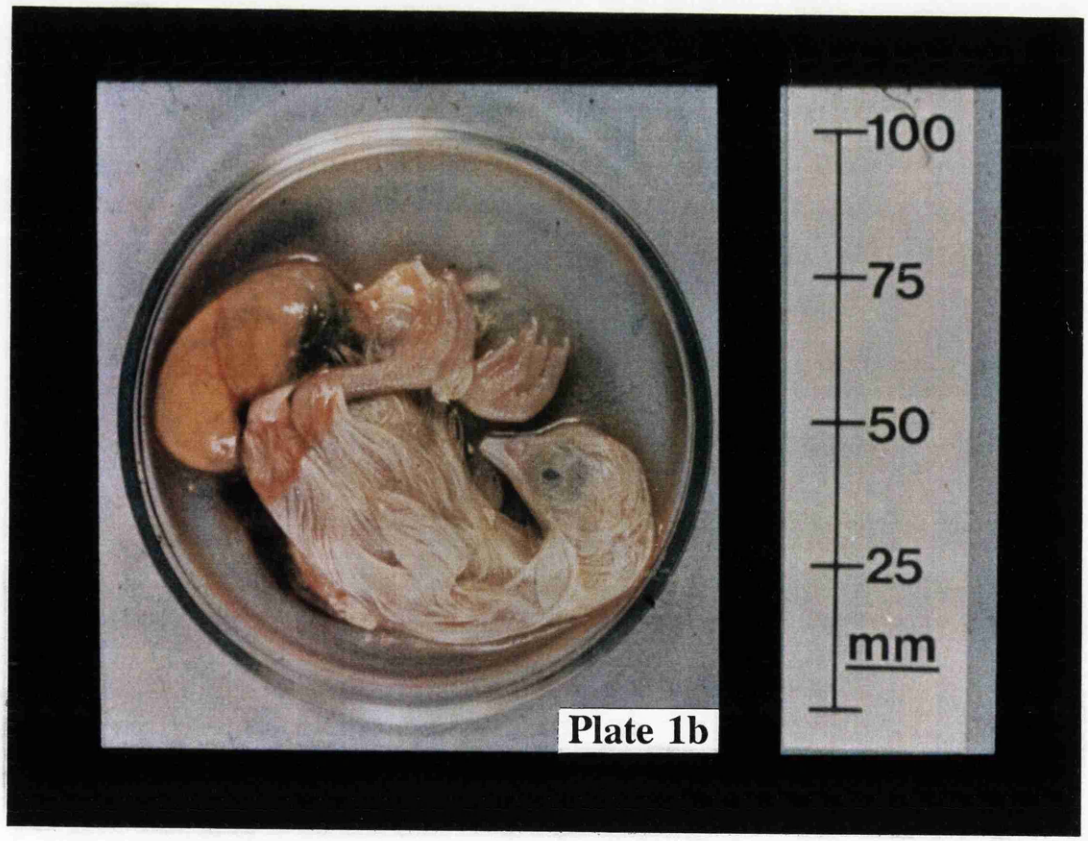


Plate 1b

brought about by nutritional and environmental circumstances, also reduce access to a host of important nutrients, other than lipids, that are required for normal development and survival and whose reduced uptake has been associated with an increase in mortality (Moore and Doran, 1962; Noble and Moore, 1966; Balnave, 1970; Singh, Weiss and Naber, 1972; Donaldson, 1981; Kuhn and Logan, 1983; Noble, Lonsdale, Connor and Brown 1986a; Noble, Shand, Connor and Brown, 1986b; Noble and Yafei, 1988; Yafei and Noble, 1990).

3.1 Development of the chick embryo.

With the onset of incubation, early embryonic development is mainly confined to the migration and reorganisation of the cells within the germinal disc (see Romanoff, 1960). Subsequent division into the ectodermal, mesodermal and endodermal regions is associated with the development of specific organs and tissues (see Romanoff, 1960; see Freeman and Vince, 1974). A more detailed description of the development of the avian embryo is obtainable in standard texts (see Romanoff, 1960; see Ede, 1978).

Although certain organs and tissues become differentiated from the fourth day of incubation, it is not until about the tenth day that the embryo takes on a recognisable appearance. In addition to the true embryonic tissues, there is an associated development of specific extra-embryonic structures during development (see Romanoff, 1960; Lambson, 1970; see Freeman and Vince, 1974), one of the primary functions of which is to facilitate substrate availability and sustain metabolism within the embryo.

Major amongst these tissues is the yolk sac membrane. In addition to its central role in overall yolk lipid assimilation, the yolk sac membrane is involved in a range of vital yolk lipid metabolic changes prior to uptake by the embryo (Noble and Moore, 1967b; Noble and Connor, 1982; Noble and Shand, 1985). The yolk sac membrane is essentially two-layered, being composed of an outer mesoderm and an inner endoderm which is continuous with the hind gut of the embryo (see Haller Von, 1958, cited by Romanoff, 1960). After only four days of incubation, the yolk sac membrane starts to undergo extensive vascularisation through the development of a network of capillaries destined to become the definitive circulation. The rapidity in the development and spread of the yolk sac membrane is such that, by the fifth day of incubation, it completely envelops the yolk (see Freeman and Vince, 1974). Although the yolk sac membrane is continuous with the endodermal cells of the embryonic gastrointestinal tract, passage of any yolk material into the intestine is prevented by a loop in the intestine which protrudes into the yolk until prior to hatching (see Freeman and Vince, 1974). All the nutrients required by the embryo must therefore be actively absorbed by the endoderm and transferred to the mesodermal blood vessels for onward transport to the embryo proper (Lee, Stavitsky and Lee, 1946; Taylor and Saenz, 1949).

3.2 Transfer of lipid from the yolk.

Unlike lipid assimilation in the small intestine of mammals, it has been established both morphologically and biochemically that yolk lipid uptake by the surrounding yolk sac membrane occurs through non-specific phagocytosis, a unique non-specific engulfment of the yolk lipid droplets involving no extracellular digestion (Lambson, 1970; Plate 2). Although various lipolytic enzymes within the yolk have been detected (Zacks, 1954), most of the evidence derived from investigations on the yolk

Plate 2

Electron micrograph of the yolk sac membrane at day 15 of incubation showing engulfment of the yolk material by the microvilli.

(see Noble and Cocchi, 1991)

L = lipid droplets

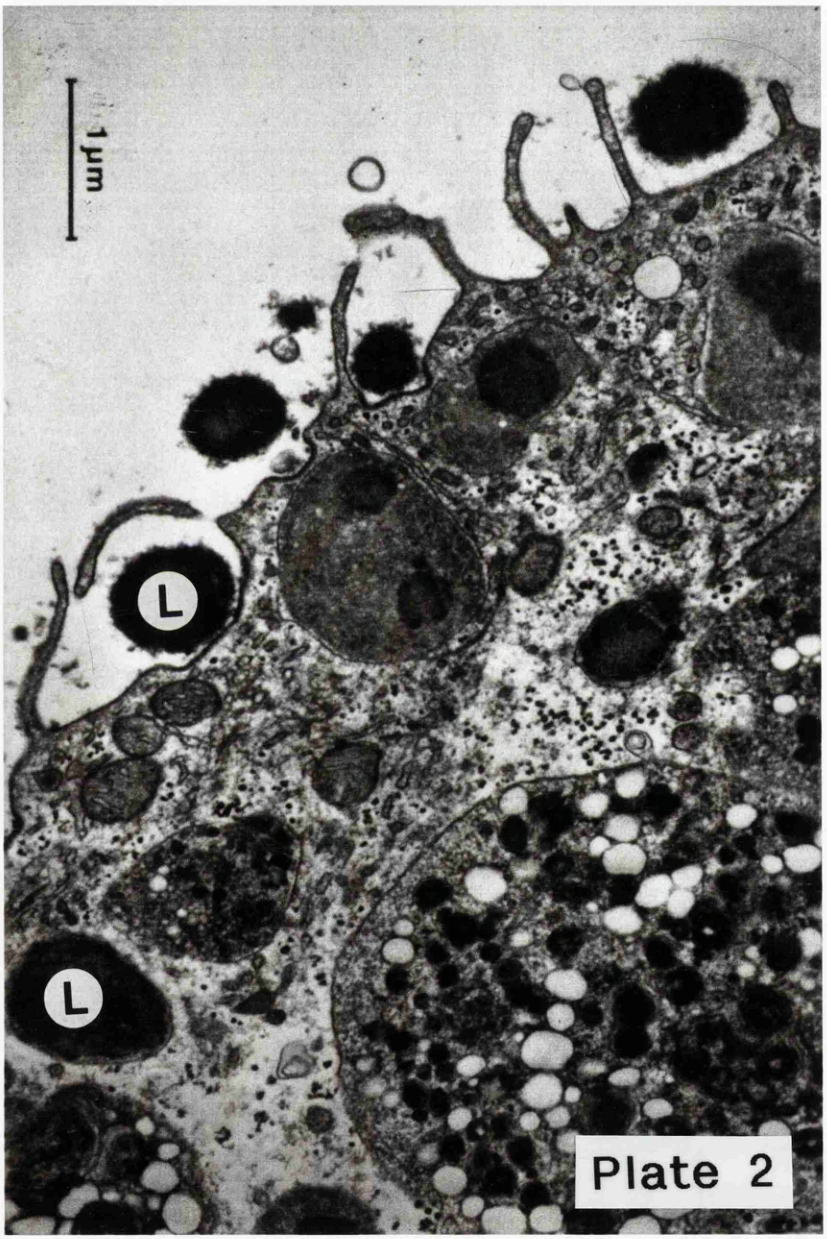


Plate 2

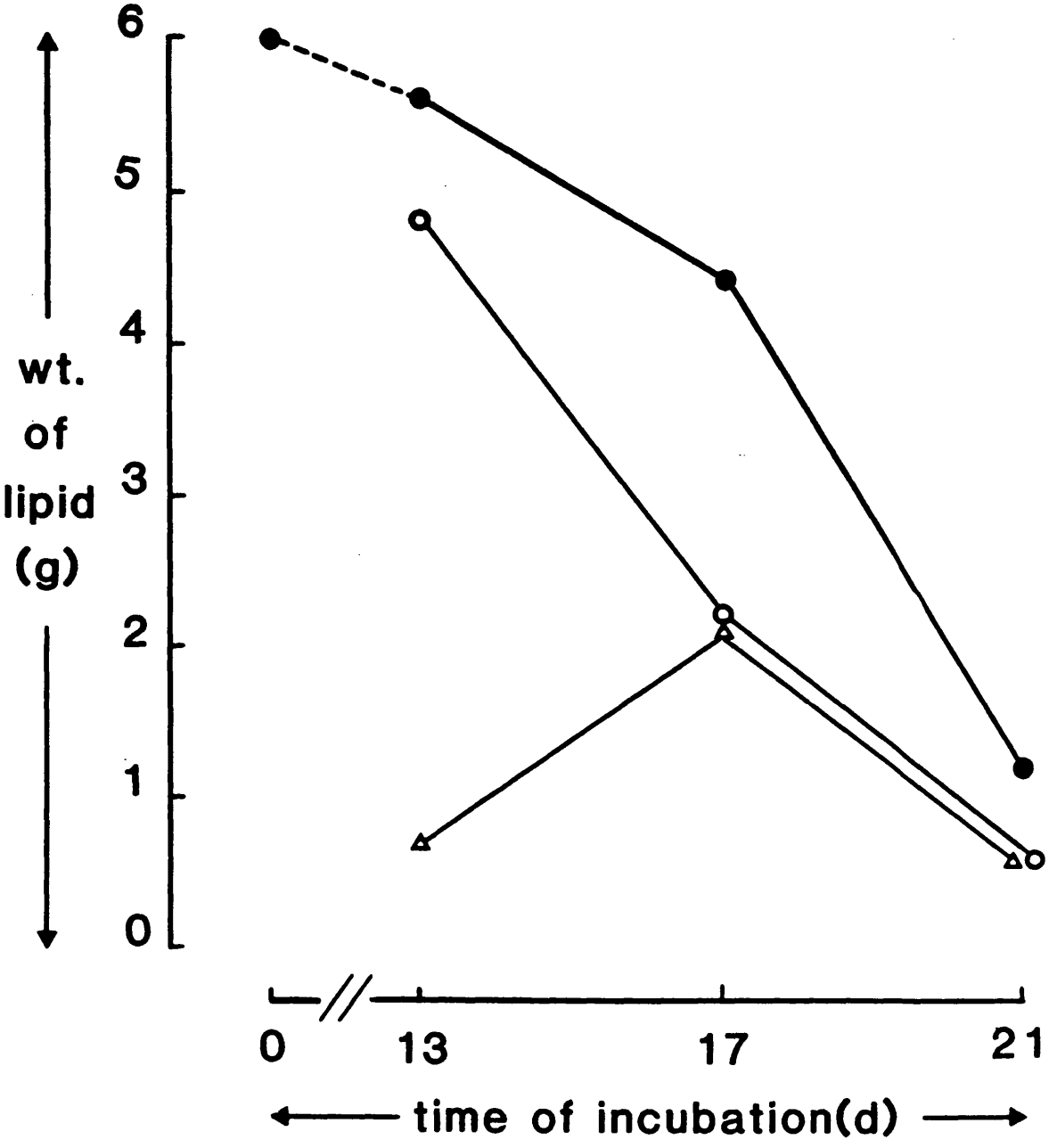
and its membrane during incubation supports the suggestion that there is no lipid breakdown before embryonic uptake (Noble and Moore, 1964, 1967b,c). Recent electron microscopic investigations (Noble and Yafei, 1988; Yafei and Noble, 1990) have supported these findings. The suggestion that lipase and protease activities exist in the endodermal cells of the yolk sac membrane, in order to facilitate the uptake of the yolk lipoprotein (Zacks, 1954; Lambson, 1970), has not been proven.

The changing distribution of total yolk lipid between the contents and the yolk sac membrane during incubation is shown in Figure 1. It can be seen that between days 13 and 17 there is considerable relocation of the yolk lipid from the contents to the membrane such that, by the seventeenth day of incubation, the yolk sac membrane contains as much lipid as the yolk (Noble and Moore, 1967b). The role of the yolk sac membrane in the uptake and temporary storage of the yolk lipid during incubation can be seen in Table 1. During the last four days of development there is a substantial decrease in the weight of lipid associated with the membrane, accompanied also by a significant decrease in the lipid content of the dry matter (Noble and Moore 1967b). This loss of lipid from the membrane coincides with a notable increase in the amount of lipid in the embryonic tissues.

Figure 1

The mean weights of total yolk (●), yolk contents (○) and yolk sac membrane (△) during development (Noble, 1986).

Figure 1



	Yolk contents			Yolk sac membrane		
Period of incubation (days)	**13**	**19**	**21**	**13**	**19**	**21**
Triglycerides	71.8	72.8	75.9	76.6	71.9	68.6
Free cholesterol	6.1	6.0	5.0	1.9	2.1	2.3
Cholesteryl esters	0.4	0.5	0.5	3.3	4.0	6.9
Phospholipids	21.4	21.1	18.2	15.7	19.4	17.5

Table 1 Compositions of the lipid (per cent of total) present in the yolk contents and yolk sac membrane at different stages of incubation (Noble and Moore, 1967b).

4 Tissue lipid composition.

4.1 Lipids of the yolk.

4.1.1 The yolk contents.

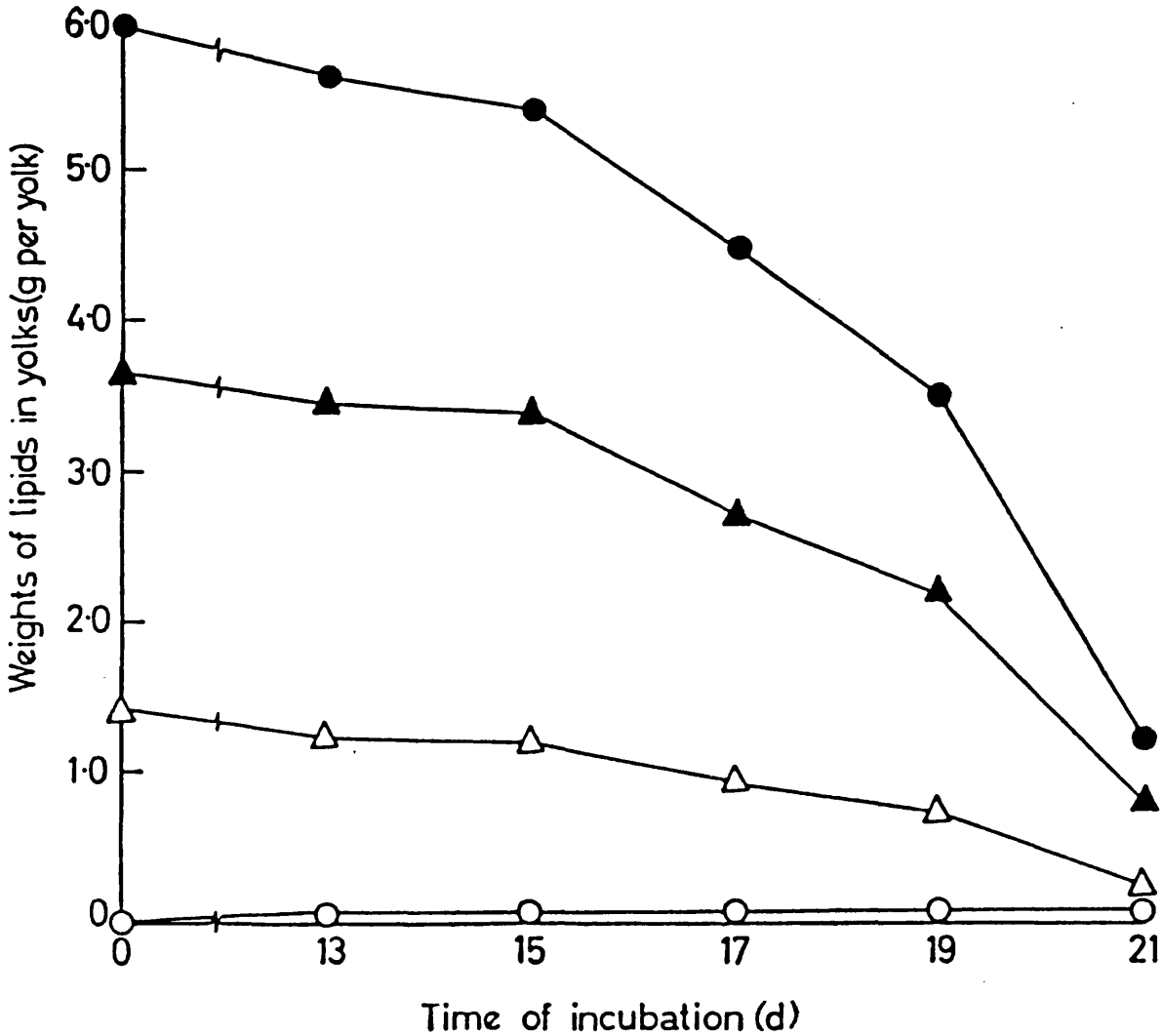
The very nature of its mode of synthesis means that there can be no 'standard' lipid composition for the yolk of any avian species. In the case of the domesticated bird, manipulation of the lipid composition of the diet is readily translated into effects on yolk lipid composition, the effects of mono- and the more common polyunsaturated fatty acids for example, are substantial (Wheeler, Peterson and Michaels, 1959; Murty and Reiser, 1961; Summers, Slinger and Anderson, 1966; Donaldson, 1967). Extremes of temperature can lead to a reduction in the overall total yolk lipid content which are not accompanied by any alteration in yolk lipid composition (Kampen Van, 1983). Nevertheless, a high degree of uniformity in egg lipid composition has been readily achieved by ensuring that the bird is provided with a stable environment, is of an age for high reproductive capacity and receives a diet that is adequate in all the necessary nutrients (Noble and Moore, 1964, 1967b). As shown from their plasma origin (Yu *et al.*, 1976), by far the principal lipid fraction of the yolk is triglyceride (71 per cent of total yolk) which is accompanied by a substantial proportion of phospholipid (22 per cent of the total lipid) and smaller amounts of free cholesterol (6 per cent). Unlike that observed in animal tissue, cholesteryl esters and free fatty acids are only minor components (Noble and Moore, 1964; Noble and Moore, 1967b; see Noble, 1986, 1987a).

As can be seen from Figure 2, the loss of total lipid from the entire yolk complex is accounted for almost entirely by a reduction in the amounts of triglyceride and phospholipid fractions (Noble and Moore, 1964, 1966, 1967b). In spite of the

Figure 2

The mean weights of total lipid (●), triglyceride (▲), phospholipid (△) and cholesterol ester (○) in the yolk at various stages of embryonic development (Noble and Moore, 1964).

Figure 2


6·0
5·0
4·0
3·0
2·0
1·0
0
Weights of lipids in yolks(g per yolk)
0
13
15
17
19
21
Time of incubation (d)

extensive loss of lipid during the last week of incubation, when expressed as percentages of the total lipid, the concentrations of the triglycerides and phospholipids show no significant changes (Moore and Doran, 1962; Noble and Moore, 1964, 1966, 1967b,c; see Noble, 1986). Low levels only of partial glycerides, free fatty acids and lyso-phospholipids have been detected during the most intensive period of yolk lipid uptake (Noble and Moore, 1964, 1966, 1967b; see Noble, 1986).

In the cholesterol ester, triglyceride and total phospholipid fractions, the fatty acid compositions remain largely unchanged from that originally associated with the egg when it was first laid. In all three lipid fractions, oleic acid is the major fatty acid present (40-45 per cent of the total fatty acids), palmitic and stearic acids together account for more than one third of the fatty acids and there is also a substantial amount of linoleic acid (Noble and Moore, 1964, 1967b,c). Phosphatidylcholine and phosphatidylethanolamine are by far the major components of the phospholipids and are characterised by differing levels of palmitic and stearic acids; the relative concentration of palmitic acid is highest in the phosphatidylcholine and lowest in the phosphatidylethanolamine (Noble and Moore, 1966, 1967c). Characteristically, there are high levels of arachidonic acid in phosphatidylethanolamine, phosphatidylserine and phosphatidylcholine, accompanied by relatively high levels of docosahexaenoic acid in the phosphatidylethanolamine (Noble and Moore, 1966, 1967c). During the last week of incubation there is a significant increase in the proportion of yolk phosphatidylcholine relative to the phosphatidylethanolamine (Noble and Moore, 1967c). Concomitant changes in the contents of more saturated phosphatidyl-ethanolamine species were noted (Noble and Moore, 1967c).

Extensive analyses on the stereospecific positional distribution of the fatty acids in the triglycerides and phospholipids (Christie and Moore, 1972; see Christie, 1982) has revealed their preferential location. In both the phosphatidylethanolamine and

phosphatidylcholine the saturated fatty acids are predominantly associated with position 1 and the unsaturated fatty acids with position 2, as is the case with most animal tissues (Hawke, 1962; Kuksis and Marai, 1967; Holub and Kuksis, 1969; Christie and Moore, 1972; see Strickland, 1973). Compared with the triglycerides of animal tissues and even the major tissues of the hen herself, the yolk triglycerides show a much greater difference in their fatty acid distributions between positions 1 and 3 (Brockerhoff, Hoyle and Wolmark, 1966). Although there are some similarities between the arrangement of the fatty acids in the yolk triglycerides and phospholipids, for example, 1-saturated-2-unsaturated species, there are also many notable differences (Christie and Moore, 1970). In particular, the triglycerides have a significant number of species with saturated fatty acids in positions 1 and 2.

Extensive studies have been made on the distribution and fatty acid profiles of the lipids associated with the specific yolk lipoproteins (see Cook, 1968; see Cook and Martin, 1969, see Gilbert, 1971; see McIndoe, 1971; Evans, Bauer, Bandemer, Vaghefi and Flegal, 1973). The major yolk lipoprotein fraction, the triglyceride-rich low density fraction, is very similar to that of mammalian plasma very low density lipoprotein and consists of a non polar core of virtually pure triglyceride stabilised at the lipid-aqueous interphase by a surrounding coat comprised of a mixture of apoproteins, phospholipids and free cholesterol (Gornall and Kuksis, 1973).

However, the lipid:protein ratio, density and size of these triglyceride-rich lipoproteins can vary considerably with changing physiological and dietary status (Martin, Turner and Cook, 1959; Bacon *et al.*, 1973; Evans, Flegal and Bauer, 1975). In general, the lipid compositions of low density fractions 1 and 2 are similar; high levels of triglycerides are associated with substantial levels of phospholipid (25-30 per cent) in which the major components are phosphatidyl-ethanolamine and phosphatidylcholine (Gornall and Kuksis, 1973; see Noble, 1987a).

The high density fraction can be separated into the α and ß lipovitellins; they also display similar lipid compositions, the major component being phospholipids, in which the proportions of phosphatidylethanolamine and phosphatidylcholine are similar to that in the triglyceride-rich low density fractions (Gornall and Kuksis, 1973). The lipid composition of the triglyceride-rich low density fraction in the granules has not been characterised to any extent, but from the limited evidence available, seems to have a composition similar to that of the low density fraction of the spheres (Gornall and Kuksis, 1973).

4.1.2 The yolk sac membrane.

Although the lipid composition of the membrane bears a great similarity to that of the yolk contents over the incubation period, it does display some notable changes as time of hatch approaches. Paramount amongst them is an accumulation of cholesteryl esters (increasing to some 4 per cent of total lipid by hatching) with a concomitant decrease in the levels of free cholesterol (Noble and Moore, 1964, 1965a, 1966, 1967b). As in the case of the yolk contents, triglycerides and phospholipids comprise by far the major lipid components of the yolk sac membrane throughout the incubation period (Noble and Moore, 1967b). The triglyceride: phospholipid ratio remains largely unchanged and similar to that of the yolk contents (Noble and Moore, 1967b; see Noble 1986, 1987b). Within the phospholipids of the membrane, there is a progressive decrease in the proportions of phosphatidylcholine relative to phosphatidylethanolamine which occurs through the selective absorption of phosphatidylethanolamine (Noble and Moore, 1967c).

Associated with these lipid changes are notable alterations in fatty acid composition. Thus, the accumulation of cholesteryl esters is accounted for by an increase in the level of cholesterol oleate (Noble and Moore, 1967b). The membrane phospholipids display higher levels of arachidonic and docosahexaenoic acids with concomitant decreases in the proportions of oleic and palmitic acids (Noble and Moore, 1965b, 1967c). The selective distribution of fatty acids in the phosphatidylethanolamine is similar to that of most animal tissues in which stearic acid in position 1 is associated with arachidonic acid or docosahexaenoic acid in position 2 (Noble and Moore, 1967c). With the exception of the C18 polyunsaturated fatty acids, no obvious changes in the fatty acid composition and positional distribution of the triglycerides in the yolk sac membrane have been found (Noble and Moore, 1964, 1967b). The suggestion that there is a preferential absorption of triglycerides containing higher levels of polyunsaturated fatty acids (Isaacks, Davies, Fergusson, Reiser and Couche, 1964) has not been confirmed (Noble and Moore, 1964, 1967b).

4.2 Plasma lipids.

The absorption of yolk lipid is associated with increasing lipid levels within the plasma of the embryo. Between days 13 and 18 there is a continuous increase in plasma lipid concentration with a slight fall in levels as time of hatch approaches (see Schjeide, 1963; Yafei and Noble, 1990). Initially, the increase in lipid level is largely accounted for by an accumulation of triglyceride-rich very low density lipoprotein. However, as incubation proceeds, the level of triglyceride in this lipoprotein is decreased and that of cholesteryl esters increased such that, just prior to hatching, cholesterol ester accounts for more than half of the total lipids present (see Schjeide, 1963; Yafei and Noble, 1990). The low density and the high density lipoprotein fractions also show enhanced cholesterol ester levels as incubation proceeds, but to a lesser extent.

Changes in the fatty acid composition of the plasma are also observed throughout the incubation period, the most significant change being the marked rise in the proportion of oleic acid (see Schjeide, 1963). This rise in oleic acid levels can be directly attributed to the appearance of cholesterol ester in which the proportion of oleic acid is unusually high (Noble, Connor and Smith, 1984; Yafei and Noble, 1990). Although there are immunological similarities between the plasma lipoproteins of the embryo and its parent (Schjeide, Rieffer, Kelly and Alaupovic, 1977), in terms of the lipid composition, the lipoproteins within the plasma of the embryo between days 13 and 21 of incubation bear little resemblance to those of the parent or those of the yolk. Alterations in the lipoprotein patterns of the embryonic plasma have been shown to subsequently affect tissue lipid uptake (Paris, Samuel, Jacques, Gache, Franchi and Aichaud, 1978; Schjeide, Kelley, Schjeide, Milius and Alaupovic, 1980).

4.3 The liver.

One of the most unusual features of the tissue lipid changes that occur in the embryo during development is that shown by the liver. By day 19 of incubation, the liver accounts for 5 per cent of the total lipid present in the embryo (see Noble 1986) but as can be seen from Table 2, its composition bears no resemblance to that of the lipid absorbed from the yolk (Noble and Moore, 1964, 1965a, 1966; Wood, 1972). Whereas, the lipid absorbed from the yolk consists mainly of triglycerides, phospholipids and free cholesterol, the accumulation of lipid during the last week of development in the liver is mainly accounted for by esterified cholesterol (Moore and Doran, 1962; Noble and Moore, 1964; 1965a; Wood, 1972; Noble, *et al.*, 1984; see Noble, 1986; see Noble, 1987b). Cholesteryl esters accumulate to such an extent that, by day 19 of incubation, they comprise 80 per cent of the total lipid of the liver and 30 per cent of the dry matter (Moore and Doran, 1962; Noble and Moore, 1964,

	CE		TG		PL	
Day of incubation	**13**	**21**	**13**	**21**	**13**	**21**
Lipid concentration mg per liver	1.63	122.0	1.07	5.39	3.63	21.5
Lipid distribution %	23.3	80.2	15.1	3.58	51.4	14.2
Fatty acid composition wt % of total						
Palmitic	2.31	1.95	23.4	23.0	22.4	17.8
Palmitoleic	0.91	0.97	1.41	1.52	<0.5	<0.5
Stearic	2.04	2.74	8.81	11.1	24.6	28.4
Oleic	77.2	79.6	30.4	35.7	7.29	7.97
Linoleic	12.7	12.5	8.32	8.23	9.94	13.8
Linolenic	<0.5	<0.5	<0.5	<0.5	1.21	1.07
Arachidonic	2.24	1.47	3.52	2.92	26.2	20.0
Docosahexaenoic	1.91	0.42	20.1	11.6	6.45	9.12

Table 2 Lipid and fatty acid compositional changes in the liver during the last week of incubation (Noble and Moore, 1964, 1966).

1966; see Noble, 1986, 1987b). The triglycerides and phospholipids which normally dominate the lipids in any liver tissue, barely rise in concentration and at hatching, account for approximately 1 and 7 per cent respectively of the total lipid present (Noble and Moore, 1964, 1966; see Noble, 1987b; see Noble and Cocchi, 1991).

Phosphatidylcholine is the major phospholipid fraction in the liver, its level never exceeds 55 per cent of the total phospholipids and shows a decline towards the end of the incubation period (Noble and Moore, 1967a, Wood, 1974). The proportion of phosphatidylethanolamine remains constant at about 30 per cent of the total phospholipid and that of phosphatidylserine at about 12 per cent (Noble and Moore, 1967a). Phosphatidylinositol is absent from the yolk and must be synthesised in the embryo.

The fatty acid composition of the liver phospholipids are characteristically different from that of the yolk at all stages of development; the phospholipids of the liver exhibit an unusually higher level of arachidonic acid compared with the yolk (Noble and Moore, 1964, 1965b, 1967a,c; Wood, 1974). The most pronounced changes in the fatty acid composition of the liver phospholipids during embryonic development occur in the phosphatidylcholine fraction and involve alterations to both saturated and unsaturated acids (Noble and Moore, 1967a, Wood, 1974; Abad, Bosch, Munico and Ribera, 1976). During incubation there are alterations in the concentration of specific intramolecular fatty acid pairings, in particular, the gradual replacement with age, of 1-palmitoyl-2 arachidonyl by 1-stearoyl-2-linoleoyl and 1-stearoyl-2-docosahexaenoyl species (Noble and Moore 1967a; Wood, 1974; Abad *et al.*, 1976). It has been suggested (Noble and Moore, 1967a) that these changes may be associated with alterations in the activities of the two principal metabolic pathways involved in the synthesis of phosphatidylcholine. In common with very much earlier conclusions (Hevesy, Levi and Rebbe, 1938; Budowski, Bottino and Reiser 1961), these later

analyses (Noble and Moore, 1967a; Wood, 1974; Abad *et al.*, 1976) confirmed that at some stage during the transfer from yolk to embryo, extensive hydrolysis and resynthesis of the phospholipids occur following yolk assimilation.

A feature of the accumulated cholesteryl esters is their very high content of oleic acid, some 70-75 per cent by weight of the total long chain fatty acids present (Moore and Doran, 1962; Noble and Moore, 1964, 1966). A surprising feature of the liver triglycerides is their unusually high concentration of docosahexaenoic acid during the final week of incubation (Noble and Moore, 1964). At day 13 of incubation, the level of docosahexaenoic acid in the triglyceride fraction is very much greater than in the phospholipid fraction with which the fatty acid is usually associated.

4.4 The bile.

The biliary lipids of the chick embryo show a unique pattern when compared with other animal species (see Portman, Osuga and Tanaka, 1975; Noble and Connor, 1984). Of the total lipid present, phospholipids account for less than half and there are substantial proportions of both cholesterol ester and triglyceride. During the final week of incubation, the proportion of cholesteryl esters increases significantly and that of the triglyceride decreases. The relative proportions of phospholipid and free cholesterol remain virtually unchanged (Noble and Connor, 1984). Associated with these lipid changes are notable alterations in fatty acid composition. In particular, the accumulation of cholesteryl esters is associated with increasing levels of oleic acid. In common with other animal species, the biliary lipids of the chick embryo contain a high proportion of linoleic acid but there are substantial levels also of arachidonic acid (Noble and Connor, 1984).

4.5 The muscle.

The major lipid fractions of the heart and skeletal muscle of the embryo are phospholipids and free cholesterol (Hsiao and Ungar, 1969; Wood, 1972; Kent, Schimmel and Vagelos, 1974). Studies on the heart tissue have shown that major changes occur in the triglyceride levels during embryonic development; these are associated with a concomitant reduction in the levels of cholesterol ester (Wood, 1972). The major components of the phospholipid fraction in the muscles are phosphatidycholine and phosphatidylethanolamine, both of which contain high levels of C20 and C22 polyunsaturated fatty acids (Kent *et al.*; 1974; Wood, 1974; Boland, Chyn, Roufa, Reys and Martonosi 1977; Nakagawa, Waku and Ishima, 1982). Phosphatidylcholine displays significantly lower levels of palmitic acid throughout the incubation period compared with phosphatidylethanolamine (Wood, 1974). The levels of saturated and monoenoic fatty acids of the heart phospholipids remain unchanged as development progresses. However, there is a dramatic increase with embryonic development in the proportion of arachidonic acid and a decrease in the level of docosahexaenoic acid (Nakagawa *et al.*, 1982). These changes are associated in the main with the phosphatidylethanolamine fraction, but also occur to a lesser extent in the phosphatidylcholine (Nakagawa *et al.*, 1982). The decrease in the proportion of docosahexaenoic acid correlates well with the decrease in heart cell mitotic index as the time of hatch approaches (see Romanoff, 1960). The relative concentration of linoleic acid increases in the heart phosphatidylcholine as development progresses (Wood, 1974).

As the time of hatch approaches, the lipid content of the skeletal muscles decreases, mainly through a reduction in the level of triglyceride (Hsiao and Ungar, 1969). Although the phospholipid composition remains relatively unchanged during development, there are significant alterations in fatty acid composition (Boland *et al.*,

1977). Between the twelfth day of incubation and hatching, the concentrations of palmitic and arachidonic acids decrease and that of linoleic increases (Boland *et al.*, 1977).

4.6 The eye and brain.

The major lipid fractions of the chick embryo brain are phospholipids and free cholesterol (Wood, 1972). As incubation proceeds, an increase in the concentration of free cholesterol is accompanied by high levels of polyunsaturated fatty acids of the n-3 and n-6 series in the phospholipid fraction (Miyamoto, Stephanides and Bernsohn, 1967; Gonzalez-Ros and Ribera, 1980; Bordoni, Cocchi, Lodi, Turchetto and Ruggeri, 1986; Anderson, Connor, Corliss and Don 1989; Noble and Cocchi, 1989a; Anderson, Connor and Corliss, 1990). Although the brain displays a relatively stable lipid composition, determinations over the incubation period (Miyamoto *et al.*, 1967; Gonzalez-Ros and Ribera, 1980; Bordoni *et al.*, 1986) have shown a specific accumulation of phospholipids, rich in polyunsaturated fatty acids, to occur particularly at around the twelfth day of incubation and associated with a build-up of polyneural discharges.

Lipids play an important role in the membrane structures of mitochondria, nuclei, synaptic vesicles and myelin in both the white and grey matter (see Chapman, 1972). The lipid classes vary from one structure to another; for example, unusual lipids such as the cerebrosides are typical constituents of myelin, whereas gangliosides are typical of synaptosome membranes (see Chapman, 1972). Overall, the brain phospholipids are characterised by extremely high but varying levels of phosphatidyl-ethanolamine and phosphatidylserine (Wood, 1974). Phosphatidylethanolamine contains much higher levels of C20 and C22 fatty acids compared with phosphatidyl-choline. Phosphatidylserine, like that of the liver and heart, contains high levels of

palmitic and stearic acids, which together account for 60-70 per cent of total fatty acids. In addition, the brain phosphatidylserine is characterised by its high level of docosahexaenoic acid.

The fatty acids also change from one region of the brain to another. Thus, phospholipids from the grey matter display a greater proportion of polyunsaturated fatty acids compared with the myelin fraction (see Chapman, 1972). The brain cells and organelles contain only trace amounts of linoleic and linolenic acids (Wood, 1974; Anderson *et al.*, 1989; Noble and Cocchi, 1989a; Anderson *et al.*, 1990) and studies have shown that arachidonic and docosahexaenoic acids are the preferred fatty acids for the differentiation and multiplication of the cells and neuromediation (see Neuringer, Anderson and Connor, 1988; Anderson *et al.*, 1989, 1990). With the exception of sphingomyelin, the phospholipids show only minor changes in their fatty acid composition during incubation; the most significant change is in the level of docosahexaenoic acid which decreases rapidly as time of hatch approaches (Wood, 1974). It has been suggested that this abrupt decrease in docosahexaenoic acid levels is accounted for by a significant turnover of this acid at the time of hatch (Wood, 1974).

The eye, in common with other nerve tissues such as the brain, also requires a specific lipid and fatty acid composition for functional development. As in the case of the brain, an accumulation of free cholesterol during incubation is accompanied by high levels of phospholipids containing large amounts of the polyunsaturated fatty acids of the n-3 and n-6 series (Zalenka, 1978; Alejandre, Marco, Ramirez, Segovia and García-Peregrin, 1984; Rintoul, Creed and Conrad, 1984; Bordoni *et al.*, 1986; Anderson *et al.*, 1989; Noble and Cocchi, 1989a; Anderson *et al.*, 1990). In contrast to the brain, high levels of polyunsaturated fatty acids are discernible in the eye from about day 10 of incubation, but their concentrations rapidly decline from

the sixteenth day of incubation and are coincident with the first behavioural response to light (Bordoni *et al.*, 1986). Studies have shown that exposure of the egg to light during the incubation period seems to affect the synthesis and subsequent concentration of essential phospholipids within the eye (Noble and Cocchi, 1989a).

4.7 The lung.

The lung of the chick embryo, like that of mammals, is rich in phospholipids. In spite of the fairly uniform rate of lipid increase in the lung during incubation (Petrik and Riedel, 1968; Tordet and Marin, 1976; Hylka and Doneen, 1982), there is a specific accumulation of phospholipids around the eighteenth day. The major phospholipid throughout incubation is phosphatidylcholine which comprises about half of the total; individual levels of sphingomyelin, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, lysophosphatidylcholine and phosphatidylglycerol are all very much lower. The rate of synthesis of phosphatidylcholine remains higher than the other phospholipids during incubation and at day 18, that is, just prior to respiration, there is a marked increase in phosphatidylcholine levels, together with sphingomyelin (Tordet and Marin, 1976; Compton and Goeringer, 1981; Hylka and Doneen, 1982). The accumulation of phosphatidylcholine is dependent to a large extent on the synthesis of the disaturated component, dipalmitoyl phosphatidylcholine (Tordet and Marin, 1976; Hylka and Doneen, 1982). Thus, the contribution of the phospholipid species to total lung concentrations in the chick embryo is similar to that occurring in mammalian species in which the overall lipid composition and its biosynthesis are directly related to the production of surfactant. However, unlike the mammalian embryo in which choline incorporation provides the major pathway of phosphatidylcholine synthesis, in the embryonic chick, both choline incorporation and the methylation of phosphatidylethanolamine function similarly throughout incubation (Compton and Goeringer, 1981).

4.8 The adipose tissue.

Extensive pads of subcutaneous tissue develop during the second half of the incubation period. These deposits occur particularly in the ventral, thoracic and thigh regions (Langslow and Lewis, 1972). With the approach of hatching, there is a sharp increase in the lipolytic activity associated with the pads (Langslow, 1972), a feature which becomes maximised immediately after hatching and the rapid utilisation of the deposits.

5 Metabolism.

5.1 Cholesterol metabolism.

During development, the compositional changes which occur within the yolk and embryonic tissues and the subsequent accumulation of very large amounts of cholesterol oleate in the liver, indicate a specific role for free cholesterol in the absorption and assimilation of yolk lipid. Since the lipids of the yolk contents contain low proportions only of cholesteryl esters with very much lower levels of oleic acid (Noble and Moore, 1967b) than subsequently deposited in the liver (Noble and Moore, 1964, 1966; Noble *et al.*, 1984), direct assimilation of yolk cholesteryl esters and incorporation into the liver is unlikely. The accumulation in the yolk sac membrane of cholesteryl esters displaying levels of oleic acid similar to that found subsequently in the liver (Noble and Moore, 1964, 1967b), the presence of oleic acid-rich cholesteryl esters in the plasma draining the yolk sac membrane (see Schjeide, 1963; Yafei and Noble, 1990) and the role of the yolk sac membrane in the assembly of the lipoprotein complexes (Lambson, 1970; Yafei and Noble, 1990), all implicate the yolk sac membrane as the source of the cholesteryl esters.

A comparative analysis has been made of the accumulation and synthesis of cholesteryl esters in the yolk contents, yolk sac membrane and liver during the last week of incubation (Noble *et al.*, 1984). In contrast to the yolk contents, the liver and yolk sac membrane both showed abilities to synthesise, *in vitro*, appreciable quantities of cholesteryl esters from free cholesterol (Noble *et al.*, 1984). The presence of an active cholesterol esterifying system within the yolk sac membrane was also indicated from measurements of the net changes in the relative proportion of cholesteryl esters before and after incubation (Noble *et al.*, 1984). Associated with the *in vitro* lipid change were notable differences in the pattern of fatty acids that were incorporated into the cholesteryl esters of the liver and yolk sac membrane. The fatty acid pattern of the cholesteryl esters synthesised by the yolk sac membrane displayed a very high level of oleic acid and was almost identical to that of the cholesteryl esters which accumulated in the liver (Noble *et al.*, 1984). The cholesteryl esters synthesised *in vitro* by the liver displayed a very much lower level of oleic acid incorporation (Noble *et al.*, 1984).

There is overwhelming evidence therefore, to substantiate the idea that the hepatic accumulation of cholesterol ester is associated with, or derived from, its synthesis in the yolk sac membrane. Some ability of the liver to make a contribution through its own ability to synthesise cholesteryl esters must exist (Noble *et al.*, 1984). Other than the brain, where cholesterol synthesis is very active and accounts for about 90 per cent of the total cholesterol content (Connor, Johnston and Lin, 1969; Svanberg, 1970; Jain, Lindahl and Svanberg, 1972; Wong and Lennarz, 1982), yolk assimilation satisfies almost all the cholesterol requirements of the embryonic tissues.

The fact that the yolk sac membrane is the major site of lipoprotein synthesis and assembly prior to passage into the embryo, together with the relatively high concentration of cholesterol oleate in the lipids of the embryonic plasma (see Schjeide, 1963), suggests that the functional role of cholesteryl esters is in the maintenance of the structure and stability of the lipoproteins, specifically involved in yolk lipid transfer (see Noble, 1987a). Indeed, studies have shown that an important function of cholesterol ester synthesis is in the maintenance of particle stability and large lipoprotein complexes (Vandenheuval, 1962). The build up of cholesteryl esters in the liver as incubation proceeds would represent therefore, an accumulation of remnant lipoproteins following yolk lipid uptake by the embryonic tissues. The ability of the yolk sac membrane to synthesise cholesteryl esters, in particular cholesterol oleate, coincides with the period of intensive redistribution of lipid between yolk contents and membrane (Noble and Moore, 1967b; Noble *et al.*, 1984). The increasing ability of the liver during incubation to synthesis cholesteryl esters containing a more conventional spectrum of fatty acids, agrees with the finding that there is a rapid dissolution of accumulated cholesterol oleate following hatching (LeGoff and Marquie, 1980).

5.2 Polyunsaturated fatty acid metabolism.

An important feature in the lipid accumulation of the chick embryo is the extensive difference between the patterns of the major C18, C20 and C22 polyunsaturated fatty acids of the yolk contents compared with those of the yolk sac membrane and the embryonic tissues. High levels of linoleic acid are consistently present in the phospholipids but between the yolk contents, yolk sac membrane, plasma and embryonic tissues, the proportions of C20 and C22 polyunsaturates increase substantially (Donaldson, 1964; Noble and Moore, 1964, 1965b, 1967a,b,c; Bordoni *et al.*, 1986; Anderson *et al.*, 1989, 1990); for example, whereas during the final

week of incubation arachidonic acid accounts for some 3 and 7 per cent respectively of the total fatty acids of the phosphatidylcholine and phosphatidylethanolamine fractions of the yolk contents (Noble and Moore, 1967c; see Noble, 1987b), the level of arachidonic acid in the same fractions of the liver exceeds 20 per cent (Noble and Moore, 1967a; see Noble, 1987b), particularly during the early part of yolk mobolisation. The relative concentration of docosahexaenoic acid in the liver phospholipids is also higher than in the phospholipids of the yolk contents. The triglycerides of the liver and to a lesser extent of several other major tissues, also contain unusually high levels of docosahexaenoic acid; in most instances, the levels of docosahexaenoic acid are significantly higher than within the phospholipids (Noble and Moore, 1964).

One of the major roles for the yolk sac membrane in yolk lipid assimilation is in the supply to the embryo of a range of polyunsaturated fatty acids at concentrations which cannot be provided by the parent bird via the original yolk. Investigations concerning the role of the yolk sac membrane on the lipid composition of the embryonic tissues (Noble and Shand, 1985) have identified appreciable Δ6-desaturation activity and the conversion of linoleic acid to arachidonic acid in both the yolk sac membrane and liver (Table 3). The membrane exhibited a particularly high level of linoleic acid conversion in the early stages of yolk lipid absorption (Noble and Shand, 1985). With the approach of hatching, desaturation activity in the membrane decreased but was increased within the liver (Noble and Shand, 1985).

Since the majority of the embryonic phospholipid arises through resynthesis (Hevesy *et al.*, 1938; Budowski *et al.*, 1961, Noble and Moore, 1967a; Wood, 1974; Abad *et al.*, 1976), the presence of an active desaturation system in the yolk sac membrane (Noble and Shand, 1985), presents an opportunity to considerably

	Liver			Yolk sac membrane		
Days of incubation	**15**	**17**	**19**	**15**	**17**	**19**
Linoleic desaturation (pmol/2hr per mg protein $x10^{-2}$)	11.1 ±0.96	13.9 ±0.79	15.7 ±1.68	28.1 ±4.93	6.11 ±0.88	7.92 ±1.32
Stearic desaturation (pmol/2hr per mg protein $x10^{-2}$)	61.7 ±6.88	19.3 ±2.95	20.9 ±4.08	39.5 ±11.2	31.4 ±1.59	17.2 ±0.48

Table 3 The Δ 6- and Δ 9-desaturase activities of the liver and yolk sac membrane during the last week of development (Noble and Shand, 1985).

enhance the arachidonic acid level of the absorbed lipid. In the case of docosahexaenoic acid, its accumulation in the embryonic tissues is accounted for both by desaturation of C18 precursors and also by the preferential absorption of yolk phosphatidylethanolamine species that are particularly rich in the acid (Noble and Moore, 1967b,c). There does not appear to be any preferential absorption from the yolk contents of phospholipid species rich in arachidonic acid (Noble and Shand, 1985). The presence of extensive Δ 9-desaturation activity and the conversion of stearic acid to oleic acid has also been identified in the yolk sac membrane. Such a process would undoubtedly make a considerable contribution to the oleic acid requirements of the cholesterol esterification process (Noble and Shand, 1985; see Noble, 1987b).

Studies have shown that there is a considerable similarity with mammalian species in the overall relationship between the polyunsaturated fatty acids supplied by the mother and those accumulated by the developing embryo (Noble and Moore, 1967a,b,c; see Noble, 1980, 1981a; Noble, Shand and Christie, 1985; see Noble and Cocchi, 1989b). Both avian and mammalian species display a similar discrepancy in the range and concentration of the major polyunsaturated fatty acids able to be supplied by the mother and those required by the embryo (Noble and Moore, 1967a,b,c; see Noble, 1980, 1981a; Noble *et al.*, 1985; see Noble and Cocchi, 1989b). In the case of the mammal, the need for higher levels of C20 and C22 polyunsaturated fatty acids is satisfied through the metabolic intervention of the placenta, that is, the specialised tissue that separates the maternal and foetal circulations (see Noble, 1980; Shand and Noble, 1979, 1981b, 1983). The synthesis of longer chain polyunsaturated fatty acids is undoubtedly in response to a specific requirement for membrane development and specialised tissue growth during the embryonic period.

6 Lipid changes in the residual yolk and liver after hatching.

In spite of the extensive absorption of yolk lipid by the chick embryo during the final week of incubation, some 28 per cent of the yolk lipid still remains unabsorbed at hatching (Noble and Ogunyemi, 1988, 1989). Following hatching, there is a rapid uptake of the residual yolk such that by day 5 to day 6 after hatching, only a very small amount remains (Entenman, Lorenz and Chaikoff, 1940; see Romanoff, 1960; Noble and Ogunyemi, 1988, 1989). The suggestion that the absorption of the majority of the lipid involves the endodermal release, via the yolk sac membrane, directly into the circulation of the chick by a process analogous to that which has occurred during embryonic development, is widely supported (Bellairs, 1963; Lambson, 1970; see Freeman and Vince, 1974; Kusuhara and Ishida, 1974). Histological observations on the chick have revealed the presence of high activities within the yolk sac membrane of enzymes necessary for absorption, suggesting the continuation of endodermal involvement well after hatching (Kusuhara and Ishida, 1974). However, there is also evidence for the direct expulsion of the remnant yolk material through the yolk stalk into the gastrointestinal tract, leading to a rapid regression of the yolk sac membrane and its function (see Romanoff, 1960). Recent observations (Noble and Ogunyemi, 1988, 1989) imply that the actual mechanism lies somewhere between these two suggestions. Following hatching, the remnant yolk complex displays a marked accumulation of cholesteryl esters and C20 and C22 polyunsaturated fatty acids (Noble and Ogunyemi, 1988, 1989), features characteristic of lipid accumulation within the yolk sac membrane (Noble and Moore, 1967b; Noble and Shand, 1985; see Noble, 1987b).

Growth of the liver after hatching is accompanied by a substantial accumulation of lipid and extensive changes in fatty acid composition away from that associated with embryo development (Noble and Moore, 1964, 1966, 1967a). The very high level

of cholesteryl esters present in chick embryonic livers (Noble and Moore, 1964, 1966; see Noble, 1987b) rapidly diminishes and is replaced by triglycerides and to a lesser extent, phospholipids (Noble and Ogunyemi, 1988, 1989). Furthermore, this alteration in lipid composition is accompanied by a sharp decline in polyunsaturated fatty acids within the triglycerides and phospholipids (Noble and Ogunyemi, 1988, 1989), indicative of the marked change in lipid metabolism of the liver, in order to cope with the chick's independent existence and the new demands being imposed upon the liver from the lipid now being absorbed from the diet.

7 The effect of parental age on lipid metabolism.

The effect of parental age on hatchability and early chick survival is well documented (McNaughton, Deaton and Reace, 1978; Garwood and Lowe, 1982; Shanawany, 1984). In the broiler industry, in order to increase chick yield from each hen, attempts are made to take eggs from very young birds. However, low hatchability and a reduction in early chick survival presents a considerable problem. Recent investigations with broiler-breeder stock (Shanawany, 1984) have shown that chick weights, minus residual yolk, from eggs of the same weight, were significantly lower from twenty-eight week-old parents when compared with embryos from mature parents. Further studies have revealed that the effect of flock age on hatchability and chick survival is associated with distinctive changes in the normal pattern of yolk mobilisation and utilisation, together with changes in aspects of metabolism of assimilated lipid within the tissues (Noble *et al.*, 1986a,b; Noble and Yafei, 1988; Yafei and Noble, 1990). Towards the end of incubation, the amount of lipid associated with the yolk and yolk sac membrane in birds from very young parents was greater than that of the embryos from mature parents and there was a much greater proportion of lipid still remaining within the yolk contents (Noble *et al.*, 1986a). At day 19 of incubation the amount of lipid associated with the extrahepatic tissues in

these embryos was also very much reduced (Noble *et al.*, 1986a). From the evidence available on gross lipid concentration and composition, it was concluded that embryos from the young parents were being denied access to sufficient yolk lipid for normal development during the final week of incubation (Noble *et al.*, 1986a).

Subsequent electron microscopic studies (Noble and Yafei, 1988; Yafei and Noble, 1990) on the lipid transfer and accumulation during the last week of incubation, confirmed such conclusions; low levels of plasma low-density lipoproteins and an alteration in the relative proportions of the major plasma lipid fractions were associated with the reduced lipid accumulations (Yafei and Noble, 1990). Associated with overall compositional changes, there are marked changes in oxidation of the assimilated lipid (Noble *et al.*, 1986b). Thus, whereas in the embryos from mature parents, hepatic oxidation of the lipids was maximum at the tenth day of incubation and decreased sharply thereafter, in the embryos from young parent stock, oxidation remained high throughout (Noble *et al.* 1986b).

8 Lipids of the subcellular organelles.

It is clear from the above that of all tissues of the chick embryo, the liver displays by far the greatest change in lipid composition during development (Noble and Moore, 1965a, 1967a; see Noble, 1986). As in other animal systems, the role of the liver is essential in all aspects of lipid metabolism (Goldman, Chaikoff, Reinhardt, Entenman and Dauben, 1950; Ranney, Chaikoff and Entenman, 1951). The possibility exists therefore, that unique and specific changes in liver lipid composition during embryonic development may have some effect on overall lipid metabolism. The intimate association of lipids and subcellular components may well involve a lipid effect at the subcellular level.

8.1 The mitochondrial lipids and metabolic function.

It is a well documented and accepted fact that the mitochondria are the power house of the cell (see Lehninger, 1964). Here are localised the enzymes of the citric acid cycle and the complex enzyme system involved in electron transmission and the subsequent release of energy in the form of high energy phosphate. Plate 3 shows a transmission electron micrograph of a mitochondrion demonstrating its structural homogeneity. As can be seen, the structure consists of pairs of membranes which give the appearance of packed sheaves or stacks of lamellae. Sometimes the membranes are branched; often, they are straight or swirled and occur in stacks or heaps, and frequently surround small vesicles. Each pair of membranes and the space they enclose averages about 140Å in width, with the space accounting for about half of this. Studies on negatively stained mammalian and plant mitochondria (Parsons 1963a,b, 1965, Parsons, Bonner and Verboon, 1965) have consistently shown a central group of membranes with tubular or vesicular projections, partly overlapped by a membrane sheet without any projections. The membrane subunits are located on the matrix side of the inner membrane (Fernandéz-Morán, 1963; Parsons, 1963a,b; Fernandéz-Morán, Oda, Blair and Green, 1964) and consist of a head, 90 Å in diameter, and a stem, 35 to 45 Å wide.

Stephens (1965) has prepared a series of electron micrographs of chick embryo liver, which show very clearly the extreme hydration of early embryonic mitochondrial matrix material. Moreover, in the young embryo, this matrix material appears to have large ' holes ' or areas of almost complete electron transparency and with age, the matrix material becomes more uniform and dense (Stephens, 1965). It has been suggested (Goldho, 1968) that the apparent absence of matrix material in the young mitochondrion may account for the very low protein: lipid ratios found in the early

Plate 3

Representative mitochondrion (M) from the chick embryo liver (13-day-old). Within the mitochondrion, the inner membrane forming the shelf-like cristae (c) clearly divides the organelles into two compartments. The inner and outer mitochondrial membranes are regularly aligned.

(Yafei, personal communication)

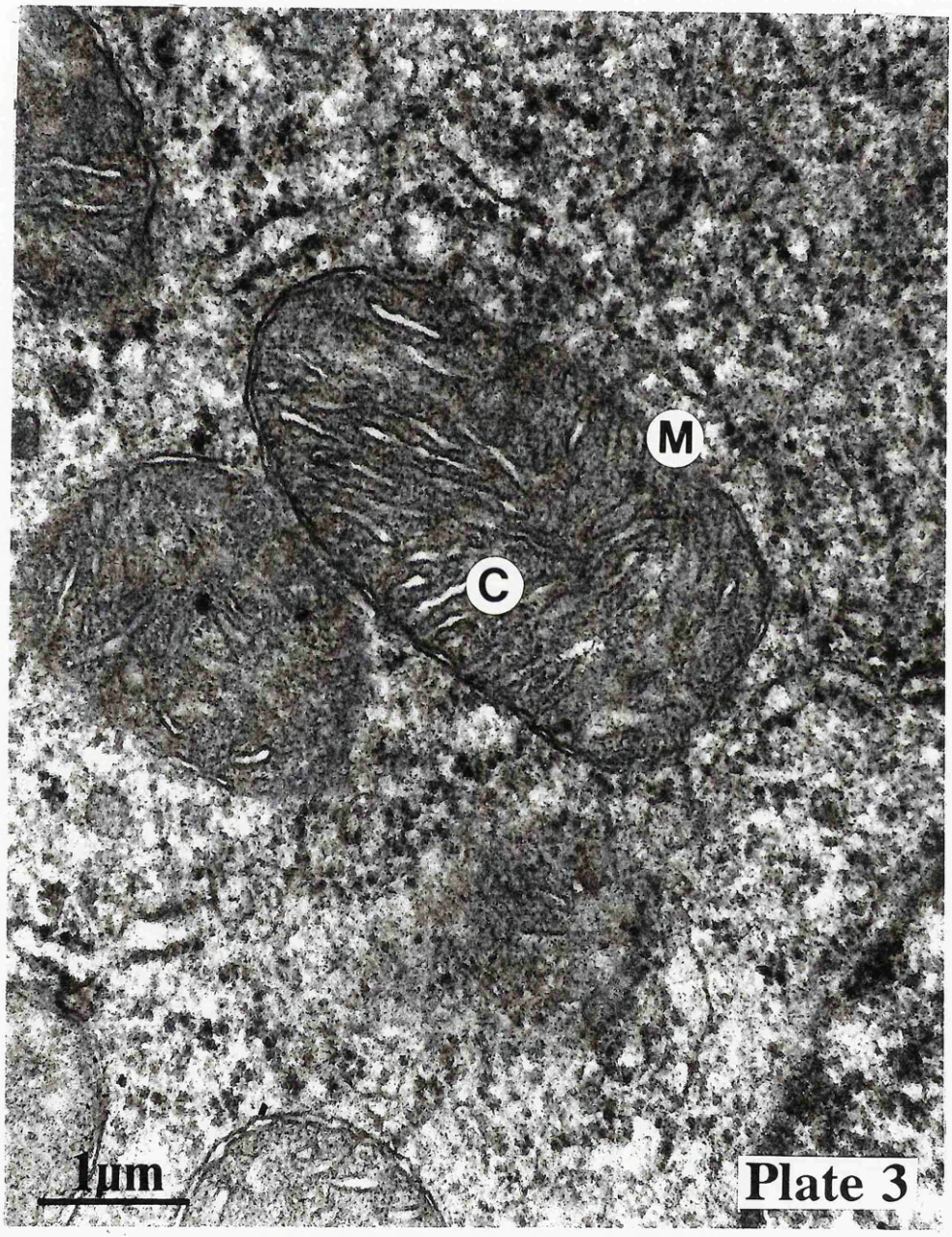


Plate 3

embryo and may indicate mitochondrial membranes of an unusually high lipid content. The finding of a relatively high protein: lipid ratio in post-hatching chicks has been explained by Goldho (1968) on the basis of two possibilities. Firstly, because most of the mitochondrial lipids are membrane bound and the contribution of the matrix is largely protein (see Lehninger, 1964), it is possible that at the post-hatching stage of development, liver mitochondria may have a large amount of matrix but relatively poor cristae development, as compared with liver mitochondria at the adult stage and secondly, that the mitochondrial membranes themselves may be relatively rich in protein at the post-hatching stage and may contribute to the high protein: lipid ratio of the whole mitochondrion.

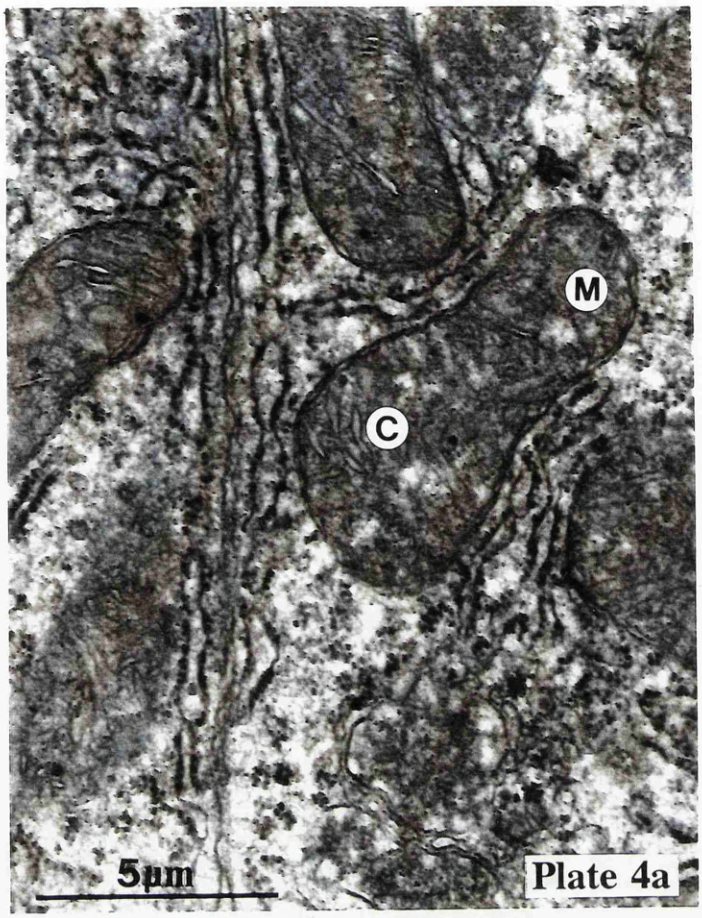
The findings reviewed by Ball and Joel (1962) on mammalian tissue, augment the observations made by Goldho (1968) that the early liver mitochondrial membranes of chick embryos have a comparatively higher lipid content compared with that of adult birds. Furthermore, these results on chick liver mitochondria (Goldho, 1968) support the suggestion that a low protein: lipid ratio is a feature typical of primitive types of vertebrate membranes such as the newly synthesised coated pinocytosis vesicles of the avian oocyte (Schjeide, Galey, Grellert, Sanlin, De Vellis and Mead, 1970). Recent studies using transmission electron microscopy (Yafei, personal communication), support these observations and in addition suggest that the morphology of the mitochondria varies with the age of embryo. In thirteen day-old chick embryos, the liver mitochondria were tubular and somewhat flattened in shape; the cristae were well developed and abundant in number (Plate 4a), but as development proceeded, the mitochondria took on a more spherical appearance; the number of cristae however, remained high (Plate 4b). Cautious interpretation of these results is necessary however, since the shape of the mitochondria may be an artifact of the preparation techniques used (see details in Yafei and Noble, 1990), or a function of the angle of orientation by the microscope.

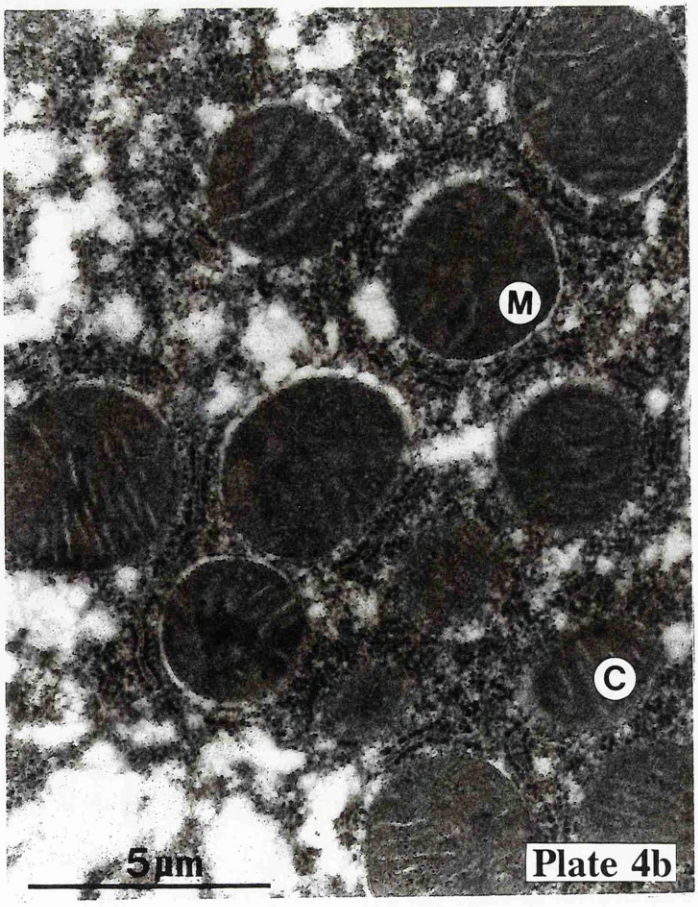
Plate 4a

Representative mitochondria (M) from the livers of 13-day-old chick embryos. The mitochondria are tubular in shape; the cristae (C) are abundant and well developed. (Yafei, personal communication).

Plate 4b

Representative mitochondria (M) from the livers of 19-day-old chick embryos. The mitochondria are spherical in shape; the cristae (C) are abundant and well developed. (Yafei, personal communication).


M
C
5µm
Plate 4a


M
C
5µm
Plate 4b

The data in the literature on the lipid composition of the particulate matter derived from mitochondria are scant since most studies have been carried out on the intact mitochondria. Furthermore, in spite of recent studies on the effect of diet on subcellular components of the chick liver (Rogel and Watkins, 1987), in general, there is very little information available on the composition of mitochondria in chick tissues; almost all the data have been obtained with mammalian species. The data are able to provide some information about the lipid composition of the mitochondrial membrane and the localised deposition of the lipid components (Harel, Jacob and Moulé, 1957). Comparisons of the data are difficult due to the wide variation of the conditions under which the studies were made. Variations, for example, exist between species (Richardson, Tappel and Cruger 1961; Getz, Bartley, Lurie and Notton, 1968) and within a species due to diet (Blomstrand and Svensson, 1974; Dewailly, Nouvelot, Sezille, Fruchart and Jaillard, 1978; Høy and Hølmer, 1979; Innis and Clandinin, 1981; Tahin *et al.*, 1981); age (Nohl and Kraemer, 1980; Vorbeck, Martin, Long, Smith and Orr, 1982); external temperature changes (Cannon, Polnaszek, Butler, Eriksson and Smith, 1975; Cherqui, Cadot, Senault and Portet, 1979) and the tissues from which the mitochondria were isolated (Getz *et al.*, 1968; Tahin *et al.*, 1981). However, the evidence concerning the effect of age on mitochondrial lipid composition in various animal tissues is conflicting (Miller and Cornatzer, 1966; Jakovcic, Haddock, Getz, Rabinowitz and Swift, 1971; Grinna, 1977; Nohl and Kraemer, 1980; Vorbeck *et al.*, 1982).

The levels of phospholipids reported to be present in the total lipids of rat liver mitochondria range from 50 to over 90 per cent (Harel *et al.*, 1957; MacFarlane, Grey and Wheeldon, 1960; Schwartz, Dreisbach, Barrionuevo, Kleschick and Kostyk, 1961). Thus Slater (1957), in a review on muscle mitochondria, reported that 79 per cent of the lipids of rat heart sarcosomes were phospholipids, whereas for

pig heart sarcosomes, a corresponding value of 91.3 per cent was found (Marinetti, Erbland and Stotz, 1958).

The availability of data on the phospholipid composition in mammalian mitochondria have served to emphasise the paucity of results available for the chick embryo. Studies have shown that the ratio of phosphatidylethanolamine to phosphatidylcholine in rat liver mitochondria is generally higher than in other membranous components (Getz *et al.*, 1968; Colbeau, Nachbaur and Vignais, 1971; Khandwala and Kasper, 1971; Zambrano, Fleischer and Fleischer, 1975) and especially higher than in the microsomes of the rat liver (Getz *et al.*, 1968; Colbeau *et al.*, 1971; Zambrano *et al.*, 1975), sheep liver and kidney (Getz *et al.*, 1968). Notable exceptions include the ovine placental tissue where the mitochondria have a lower amount of phosphatidylethanolamine (plus phosphatidylglycerol), compared with the microsomal fraction (Shand and Noble, 1984).

The levels of phosphatidylinositol and phosphatidylserine in mitochondria are generally lower than in other membranous components (Colbeau *et al.*, 1971; Khandawala and Kasper 1971; Zambrano *et al.*, 1975) and the amount of sphingomyelin varies with the type of tissue (Getz *et al.*, 1968; Shand and Noble, 1984). Compared with other membranes, mitochondrial phospholipids are usually rich in diphosphatidylglycerol (Getz *et al.*, 1968; Dennis and Kennedy, 1972; Zambrano *et al.*, 1975) which has a strong affinity for the divalent cations calcium and magnesium (Nielson, 1971), suggesting that it may exist in the form of salts or chelation complexes with cations. This suggestion is reinforced by evidence that the phospholipids play a physiological role in the passive binding of cations (Jacobus and Brierley, 1969; Scarpa and Azzone, 1969). Analysis of the fatty acid composition of diphosphatidylglycerol has revealed an extraordinarily high percentage of unsaturated fatty acids (about 90 per cent), due mainly to the high level of linoleic

acid (Colbeau *et al.*, 1971). Evidence has been presented to show that the major portion of mitochondrial diphosphatidylglycerol (75- 90 per cent) is located on the matrix side of the mitochondrial inner membrane (Nilsson and Dallner, 1977; Krebs *et al.*, 1979; Harb *et al.*, 1981).

The results obtained for the preferential distribution of phosphatidylcholine however, vary (Nilsson and Dallner, 1977; Krebs *et al.*, 1979; Harb *et al.*, 1981). Studies have shown that the majority of phosphatidylethanolamine is located on the matrix side of the inner membrane (Marinetti *et al.*, 1976; Krebs *et al.*, 1979), whereas studies on pig heart mitochondria (Harb *et al.*, 1981) have revealed that 20 to 30 per cent of the phosphatidylethanolamine is buried in the inner membrane and is therefore not accessible to the phospholipases. Similarly, Nilsson and Dallner (1977) found 85 per cent of total phosphatidyinositol to be protected against phospholipase A_2 in rat livers. In the inner mitochondrial membrane, the rate of lipid transition is extremely slow (Rousselet, Colbeau, Vignais and Devaux, 1976). The ratio of phosphatidylcholine to phosphatidylethanolamine for outer membranes is generally high, (3 per cent) approaching that found in both mammalian and avian microsomes (Ward and Pollak, 1967; Getz *et al.*, 1968), whereas for inner membranes, the proportions are lower and similar to that of whole mitochondria (Lévy and Sauner, 1968; Stoffel and Schiefer, 1968; McMurray and Dawson, 1969).

Mitochondrial lipids are particularly rich in polyunsaturated fatty acids. Their levels in relative proportion vary with tissue and thus, whereas linoleic and arachidonic predominate in heart and liver (Palmer, Schmid, Pfeiffer and Schmid, 1981; Tahin *et al.*, 1981), docosahexaenoic acid is the principal component in the brain (Tahin *et al.*, 1981). Contradictory evidence exists concerning the fatty acid distribution in mitochondrial membranes. The fatty acid compositions of the outer and inner mitochondrial membrane lipids were similar in rat liver (Stoffel and Schiefer, 1968;

Colbeau *et al.*, 1971) and guinea pig liver (Parkes and Thompson, 1970), suggesting that the minor differences in chain length and degree of unsaturation could be due to the phospholipid pattern, rather than the spacial distribution of fatty acids in the mitochondrial membranes and that each phospholipid had a distinctive fatty acid composition more or less similar in all membranes. In contrast to these observations, the finding of increased amounts of unsaturated fatty acids in the inner mitochondrial membranes of rat liver (Huet, Lévy and Pascaud, 1968) and pig heart (Comte, Maïsterrena and Gautheron, 1976) has been described. The outer membranes are three-fold richer in total phospholipids and six-fold richer in cholesterol than the inner membranes, based on protein (Lévy and Sauner, 1968; Parsons and Yanu, 1967).

Areas of specific contact between the inner and outer mitochondrial membrane have never been characterised nor have they been isolated. Nevertheless, studies on phospholipid transfer in mammalian (Parkes and Thompson, 1970) and yeast cells (Daum and Paltauf, 1984) indicate that cytosolic proteins are able to catalyse a transfer of diphosphatidylglycerol between cellular membranes. The exchange of phospholipid species between the mitochondria and other cellular components is well recognised in a range of animal species (Wirtz and Zilversmit, 1968; McMurray and Dawson, 1969; Wirtz and Zilversmit, 1969); In particular, this exchange appears to involve the outer membrane (Wojtczak, Baranska, Zborowski and Drahota, 1971), although the evidence for this is conflicting (Blok, Wirtz and Scherphof, 1971; Sauner and Lévy, 1971; Wojtczak *et al.*, 1971).

Usually, the level of sterols in mammalian mitochondria is low (Spiro and McKibbin, 1956; Schwartz *et al.*, 1961); exceptions include mitochondria from the gastric mucosa of several animal species (Sen and Ray, 1980), from rat heart (Palmer *et al.*, 1981) and from ovine placental tissue (Shand and Noble, 1984), all of which contain increased amounts of cholesterol.

Triglycerides have been detected in rat liver mitochondrial membranes (Colbeau *et al.*, 1971) in spite of the fact that neutral glycerides are not considered to be structural elements of biological membranes.

Functions of the mitochondrial lipids.

The functions of mitochondrial lipids are described in detail in several review articles (see McMurray, 1973; see Daum, 1985). Their functions are too numerous to be mentioned in entirety but nevertheless, the following few examples are an attempt to summarise the present knowledge available on the role of mitochondrial lipids and highlight their importance.

Studies, mostly undertaken on bovine heart, have confirmed that changes in the mitochondrial lipid pattern have a marked effect on respiration and energy production. It has been shown that exposure of submitochondrial particles to phospholipases impairs both the rate of oxidation and phosphorylation (Burstein, Loyter and Racker, 1971a,b). The inhibitory effect of phospholipase A_2 (where possible, the standard Enzyme Commission abbreviations for those enzymes mentioned in the text, are noted in the Abbreviation's section) was prevented or reversed by bovine serum albumin, only when the enzyme concentration was low (Burstein *et al.*, 1971a,b). With respect to the role of phospholipids in mitochondrial function, it has been shown that their binding to unilamellar liposomes led to a decrease in electron transfer activity, which could be attributed to an increase in the average distance between integral proteins in the fused membranes (Schneider, Lemasters, Höchli and Hackenbrock, 1980a,b; Schneider, Lemasters and Hackenbrock, 1982).

Lipids influence the activity of mitochondrial enzymes both *in vivo* and *in vitro*. The activities of certain enzymes, most notably succinoxidase and segments of various multienzyme systems were almost totally inactivated when the mitochondria were depleted of phospholipids (Brierley, Merola and Fleischer, 1962); activities were restored by the addition of micellar suspensions of mitochondrial phospholipids and were accompanied by phospholipids binding to the lipid depleted membranes (Brierley *et al.*, 1962). Several different phospholipids have been shown to be capable of restoring cytochrome *c* oxidase activity with their effectiveness differing according to their affinity and degree of specificity for the enzyme (Robinson, Strey and Talbert, 1980). Evidence has been presented to show that the oligomycin-sensitive adenosine triphosphatase binds diphosphatidylglycerol (Santiago, Lopéz-Moratalla and Segovia, 1973) and phosphatidylserine (Brown and Cunningham, 1982). An inhibition of oligomycin-sensitive adenosine triphosphatase activity has been attributed to the presence of phosphatidylethanolamine (Brown and Cunningham, 1982).

Certain phospholipids have been found to promote enzyme activity (Bruni, Van Dijck and De Gier, 1975; Pitotti, Dabbeni-Sala and Bruni, 1980); chain length and degree of unsaturation of the fatty acid present were also shown to be important (Bruni *et al.*, 1975; Pitotti *et al.*, 1980). Acyl Coenzyme A: Sn-glycerol-3-phosphate acyltransferase activity in rat liver was found to be inhibited by diphosphatidylglycerol (Kelker and Pullman, 1979), whereas other enzymes such as mitochondrial P-450 were activated (Hall, Watanuki, Degroot and Rouser, 1979; Lambeth, 1981). The enzyme D-3-hydroxybutyrate has a strong affinity for phosphatidylcholine which is necessary for maximum activity (Isaacson, Deroo, Rosenthal, Bittman, McIntyre, Bock, Gazzotti and Fleischer, 1979). The activity of D-3-hydroxybutyrate was enhanced by unsaturated fatty acids, whereas cholesterol had an inhibitory effect (Sekuzu, Jurtshuk, Jr. and Green, 1963; Gotterer, 1967;

Lēvy, Joncourt and Thiessard, 1976). The results from several studies involving the use of synthetic phosphatidylcholine analogues (Isaacson *et al.*, 1979) and plasmalogens (Grover, Slotboom, De Haas and Hammes, 1975) suggest that the hydrophobic moiety of phosphatidylcholine is essential for lipid-protein interaction; specificity for the diacylglyceride does not exist.

Lipids also influence the stability and osmotic behaviour of mitochondria. Enrichment of media with free sterols by *in vitro* incubation with plasma lipoproteins has been shown to lead to an increased stability of rat liver mitochondria (Graham and Green, 1970). Furthermore, under conditions of severe hypotonic shock, the outer membrane of normal mitochondria ruptured in contrast to the outer membrane of cholesterol-enriched mitochondria, which remained intact. Mild hypotonic shock also had less effect under conditions of cholesterol enrichment (Graham and Green, 1970). However, conflicting evidence is provided by McLean-Bowen and Parks (1982) in studies on yeast mitochondria. Osmotic stability of yeast mitochondria was little influenced by their sterol content, although the plasma membranes from the same strains were more fragile when sterol deficient (McLean-Bowen and Parks, 1982).

Bovine heart mitochondria, depleted of phospholipids by treatment with organic solvents, not only lost their osmotic characteristics, but their ability to bind cations (Scarpa and Azzone, 1969). These functions were restored upon the addition of phospholipids, especially phosphatidylethanolamine (Scarpa and Azzone, 1969).

An influence of mitochondrial lipids on ion transport and permeability has also been demonstrated (Burstein *et al.*, 1971a; Pfeiffer, Schmid, Beatrice and Schmid, 1979; Palmer and Pfeiffer, 1981). Partial restoration of calcium ion translocation was achieved by addition of egg phosphatidylcholine or large amounts of bovine

serum albumin to bovine heart mitochondria deficient in phospholipids (Burstein *et al.*, 1971a). Whereas ion transport in rat liver mitochondria was unaffected by the introduction of unsaturated fatty acids (Pfeiffer *et al.*, 1979), in yeast strains deficient in fatty acid desaturase, the ability for active cation transport was lost when the concentration of fatty acids decreased beyond a certain level (Haslam, Spithill, Linnane and Chappell, 1973). In addition, the coupling of phosphorylation to respiration was lost and there was an increase in passive permeability of mitochondria to protons (Haslam *et al.*, 1973; Haslam and Fellows, 1977).

Observations on artificial membranes using X-ray diffraction (Rand and Sengupta, 1972) have noted that lipids can affect the membrane structure, phase behaviour and lipid-protein interaction. Diphosphatidylglycerol for example, can undergo phase transitions in the presence of polyvalent cations (Rand and Sengupta, 1972; Cullis, Verkleij and Ververgaert, 1978; see Verkleij, 1984) and as a consequence, may induce a change in the permeability properties and thus affect ion transport.

The presence of very high amounts of polyunsaturated fatty acids in mitochondrial membranes and the tendency to maintain a high degree of unsaturation under most circumstances, support the concept that polyunsaturated fatty acids play an important role in the maintenance of structural integrity of the membranes. Direct evidence for the disruption of the mitochondrial membrane during fatty acid deficiency has been demonstrated (Levin, Johnson and Albert, 1957; Hayashida and Portman, 1960a,b).

8.2 The microsomal lipids and metabolic function.

Unlike mitochondria, the term 'microsomes' does not refer to a discrete cytoplasmic organelle. It is an operational term which denotes the particulate fraction that sediments upon centrifugation at speeds in excess of 10, 000g (see Duve De, 1964; see Reid, 1967). Thus, the term microsomes often refers to the mixture of membranous elements present either as separate entities, or as complexes. These structures are derived from the smooth (or agranular) and the rough (or granular) endoplasmic reticulum. During chick development, the rough-membrane fraction contains more than 60 per cent of the proteins, ribosomal nucleic acid and phospholipids of the microsomes; glucose 6-phosphatase is present mainly in the rough-membranes, whereas adenosine triphosphatase is found predominantly in the smooth-membrane fraction (Pollak and Ward, 1967).

As for the mitochondrial studies, the chemical composition and the enzymic activities of microsomes are mainly confined to mammalian species (Dallner, Siekevitz and Palade, 1966a,b; Getz *et al.*, 1968; Giusto and Bazan, 1979; Tahin *et al.*, 1981; Cook, 1982; Shand and Noble, 1984; Innis, 1986); little data are available for the chick. Depending upon conditions of separation, microsomal fractions can exhibit a range of structural and biochemical properties; additionally, features that include age (Schjeide, Prince, Nicholls and Wanamacher, 1973; García-Gonzalez, Alejandre, García-Peregrin and Segovia, 1986a; Bordoni, Biagi, Turchetto and Hrelia, 1988), type of tissue (Getz *et al.*, 1968), diet (Tahin *et al.*, 1981; García-Gonzalez, Alejandre, García-Peregrin and Segovia, 1986b; Rogel and Watkins, 1987) also have an effect. As in the case of the mitochondria, the microsomes of the early chick embryo have been shown to display a considerably

low protein: lipid ratio and there is an increase in the level of unsaturated fatty acids towards hatching (Boland, Martonosi and Tillack, 1974).

The percentage composition of the major phospholipid classes in the chick liver microsomes (Ward and Pollak, 1967) agrees well with the results obtained for livers from a selection of mammalian species (Dallner *et al.*, 1966a,b; Getz *et al.*, 1968). Microsomes generally have a high concentration of phosphatidylcholine, the proportion being somewhat greater than that displayed by either the whole tissue or mitochondria (Ward and Pollak, 1967; Getz *et al.*, 1968; Shand and Noble, 1984). The concentration of phosphatidylethanolamine is similar to that of whole tissue (Getz *et al.*, 1968; Shand and Noble, 1984) and, in general, higher than the mitochondria (Getz *et al.*, 1968). The concentration of sphingomyelin varies, depending on the species and type of tissue but is in general low (Getz *et al*, 1968), notable exceptions being the microsomes from ovine placental tissue (Shand and Noble, 1984), sheep brain (Getz *et al.*, 1968) and chick livers (Ward and Pollak, 1967).

Substantial data exists with respect to the phospholipid composition of the rough and smooth microsomal fractions in mammals (Glaumann and Dallner, 1968; Bollen and Higgens, 1980). The limited data available for the chick embryo (Ward and Pollak, 1967) would indicate that distribution differences exist between the smooth and rough microsomes, in particular the former, showing markedly lower levels of phosphatidylcholine and higher levels of sphingomyelin.

Studies on the liver of chick embryos have shown that the amount of the membranous endoplasmic reticulum present increases considerably from day 6 of incubation (Karrer, 1960; Pollak and Shorey, 1967; Pollak and Ward, 1967). Transmission electron microscopic studies (Schjeide *et al.*, 1973) have shown that the microsomes

of liver cells of chick embryos at ten days of incubation, are morphologically similar to those present after hatching, that is, they consist predominantly of rough-endoplasmic reticulum. As hatching approaches, distinct morphological changes have been observed in the endoplasmic reticulum. Thus, the lumen becomes distended and free ribosome clusters become sparce (Schjeide *et al.*, 1973).

Qualitative changes in both neutral and complex lipid moieties have been observed in a selection of tissues during embryonic development in a range of animal species (Ward and Pollak, 1967; Glaumann and Dallner, 1968). As in the case of the mitochondria, it has been shown that the importance of the polyunsaturated fatty acids is bound up in metabolic function and particular fatty acid combinations (Palmer *et al.*, 1981; Tahin *et al.*, 1981).

Functions of the microsomal lipids.

Studies, mainly undertaken on mammalian systems, have shown that the endoplasmic reticulum is the site for the elongating fatty acid enzymes and the Δ9-, Δ6-, Δ5- and Δ4-desaturases involved in the synthesis of polyunsaturated fatty acids (Cook, 1982; see Brenner, 1984). Assembled to the Nicotinamide Adenine Dinucleotide (reduced)-cytochrome *b* microsomal electron transport chain, they play an important part in the control of the polyunsaturated fatty acid composition of the microsomes and therefore, on the function of fatty acid dependent structures, in particular membrane fluidity. There is evidence to suggest that alterations in microsomal membrane fluidity, brought about by changes in desaturation activity, antagonised membrane structure and function by activating or deactivating double bond production (Brenner, Garda, De Gómez Dumm and Pezzano, 1981; Castuma and Brenner, 1983). However, there was no uniformity in the pattern and mechanism through which this occurred. In contrast, the major rate controlling enzymes in

cholesterol biosynthesis and its subsequent conversion to cholesterol ester seemed to be controlled *in vivo* by independent mechanisms which did not appear to be modified by the physical properties of the microsomal lipid (Innis, 1986). A specific phospholipid requirement for glucose-6-phosphatase activity has been demonstrated in rat liver microsomes (Duttera, Byrne and Ganoza, 1968).

Studies have suggested that the phospholipids may play an important part in the binding of certain enzymes to the microsomal membranes (Ward and Pollak, 1967) and in the transportation of enzymes and other components (Martonosi, Donley and Halpin, 1968; Boland *et al.*, 1974). The components of the electron transport system are also dependent on phospholipids (Jones and Wakil, 1967). The activities of certain enzymes, most notably Nicotinamide Adenine Dinucleotide (reduced): cytochrome *c* oxidoreductase, for example, were reduced by the removal of the majority of the phospholipids (Jones and Wakil, 1967). An investigation into the lipid requirement for fatty acid, hydrocarbon and drug hydroxylations has shown that the transfer of electrons from Nicotinamide Adenine Dinucleotide Phosphate (reduced) to the haemoprotein (P-450) was dependent on the microsomal phospholipids (Strobel, Lu, Heidema and Coon, 1970). Moreover, there was a specific requirement in this mechanism for phosphatidylcholine (Strobel *et al.*, 1970).

Information on the molecular structure of lipid is lacking. Nevertheless, the complex interactions of lipids in the microsomes with other essential biochemical components have been detailed (see McMurray, 1973; see Brenner, 1984).

8.3 Cytosolic (Homogenate and Supernatant) lipids in the cellular supernatant.

There is a considerable paucity of information available on the homogenate and supernatant fractions of chick embryo livers; as in the case of the mitochondria and microsomes, most of the studies have been confined to mammalian systems (Getz *et al.*, 1968; Shand and Noble, 1984). Analysis of the supernatant fraction from the same tissue revealed very high levels of cholesteryl esters and triglycerides compared with the homogenate, whilst a lower proportion of phospholipids was noted (Shand and Noble, 1984).

Recent studies on chick embryos have shown that towards the end of incubation, large cytoplasmic globules accumulate in the liver (Noble, Yafei and Tullett, 1988; Yafei and Noble, 1990). Analysis of the globules (see Schjeide, 1963) has shown them to contain a very high level of oleic acid-rich cholesteryl esters which, towards the end of the incubation period, account for more than 80 per cent of the total lipid present.

Studies on ovine placental tissue (Shand and Noble, 1984) have shown that the homogenate, like that of the mitochondria and microsomes, displayed a high proportion of phospholipids (Shand and Noble, 1984). In contrast to the mitochondria and microsomes, the level of cholesteryl esters was considerably higher in the homogenate fraction (Shand and Noble, 1984). The main lipid component reported to be present in both the homogenate and supernatant fractions of various mammalian tissues is phospholipid, the major moiety of which is phosphatidylcholine (Getz *et al.*, 1968; Shand and Noble,1984).

9 Polyunsaturated fatty acids, peroxidation and function in tissue metabolism.

Tissue and subcellular components containing high levels of polyunsaturated fatty acids are highly susceptible to autoxidation. Lipid peroxidation can be stimulated in isolated cells or tissues and in suspensions of intracellular organelles by a variety of procedures, such as the addition of transition elements, ascorbic acid, cysteine and glutathione (Hunter, Gebicki, Hoffsten, Weinstein and Scott, 1963; Hunter, Scott, Hoffsten, Gebicki, Weinstein and Schneider, 1964a; Hunter, Scott, Weinstein and Schneider, 1964b; McKnight and Hunter 1966). The presence in the brain and liver of substantial levels of transition elements and polyunsaturated fatty acids, renders them susceptible to continual damage. In addition, the vulnerability of the brain is increased because it has a low antioxidant level and is surrounded by cerebral spinal fluid containing limited iron binding capacity (Stocks, Gutteridge, Sharp and Dormandy, 1974; see Halliwell and Gutteridge, 1985).

The process of lipid peroxidation can be quite specific depending upon the particular inherent structure of the membranes. There are nevertheless, general phenomena intrinsic to any membrane structure rendering them open to peroxidation. Few studies have been devoted to the peroxidation of lipids in the mitochondria and microsomes, but the reaction kinetics of lipid peroxidation have been detailed (see Logani and Davies, 1980; see Vladimirov, Olenev, Suslova and Cheremisina, 1980; see Frankel, 1982, 1985). In summary, lipid peroxidation is a free-radical-mediated process which leads to the degradation of polyunsaturated fatty acids to a complex variety of products (see Mead, 1976; see Esterbauer, 1982). Amongst the studies on lipid peroxidation in biomembranes, most have centred on peroxidative reactions of arachidonic acid which can result, both in alterations to membrane structure and

function and also interference in the synthesis of derived compounds such as the prostaglandins and leucotrienes (see Esterbauer, 1982).

Most of the data on the oxidation of lipids in subcellular membranes have involved studies on mammalian systems. The rate of lipid peroxidation in biological membranes is dependent on the rate of diffusion and collision of radicals and other interfering agents with the fatty acid chains in the lipid bilayer. Although peroxidation is greatly influenced by lipid bilayer fluidity, studies have shown that the transition temperature of the microsomal membranes is relatively constant. Thus, their phospholipids are in a fluid state within a reasonable range of temperatures (Mabrey, Powis, Schenkman and Tritton, 1977; Brenner *et al.*, 1981).

Another aspect of the influence of membrane structure on lipid peroxidation which is of significance to the mitochondria and microsomes, is the presence of substrates with catalytic properties, for example, haemoprotein and non-haeme iron, which promote the susceptibility of polyunsaturated fatty acids to peroxidation. The action of these 'chaotrophic agents' on mitochondria is through activation of lipid peroxidation and subsequent alteration in membrane structure (Hunter *et al.*, 1963; 1964a,b; McKnight and Hunter, 1966; Hanstein and Hatefi, 1970; Hatefi and Hanstein, 1970). Studies on the possible cyclic activation of mitochondrial respiration have shown that ferrous salts did not initiate oxygen uptake in mitochondrial suspensions which had been given α-tocopherol to prevent lipid peroxidation (Olenev, Suslova and Vladimirov, 1974); this was attributed to autoxidation of ferrous ions with molecular oxygen. However, ferric ions did activate respiration, although the effect was one-tenth as high as that observed when calcium ions were added (Olenev *et al.*, 1974). Studies on the binding and reduction of iron by the mitochondrial respiratory chain have revealed a possible mechanism of peroxidation control through mechanisms involving the action of ferric ions on

biomembrane function (Barnes, Conelly and Jones, 1972). It has also been suggested that the action of ferric ions was through the direct involvement in the interplay between the oxidation/reduction mechanism (Barnes *et al.*, 1972).

An increase in the membrane surface charge brought about by lipid peroxidation, has been shown to affect ion conductivity of the intrinsic bilayer (Ohki and Goldup, 1968) and the absorption of charged proteins, leading to an alteration in membrane permeability and an effect on membrane stability (Antonov, Korepanova and Vladimirov, 1976). An increase in membrane proton conductivity in mitochondria under conditions of lipid peroxidation has also been demonstrated (Putvinsky, 1977).

Uncoupling of oxidative phosphorylation is clearly associated with lipid peroxidation, involving increased proton permeability and decreased membrane stability. On the morphological side, lipid peroxidation gives rise to mitochondrial swelling (Hunter *et al.*, 1963; 1964a) and the efflux of matrix constituents from the swollen organelles; these may in turn aggravate lipid peroxidative mechanisms. Under *in vivo* conditions, the effects of mitochondrial swelling on cell viability is poorly understood.

9.1 The mode of action of antioxidants.

It is clear from what has been mentioned above that polyunsaturated fatty acids and their interrelationships play an important role during the development of the chick embryo at both cellular and subcellular levels. If this role is to be adequately promoted, then it is essential that polyunsaturated fatty acids are given adequate protection from peroxidation. A range of agents able to perform this function are present within the egg when laid, the most notable being vitamin E (of which the most important is α-tocopherol). Extensive investigations have been made into the

mode of action of many of the neutral antioxidants. The range of known components involved in the prevention of lipid peroxidation include the following: vitamin C (Niki, Tsuchiya, Tanimura and Kamiya, 1982; Bendich, Machlin, Scandurra, Burton and Wayner, 1986); vitamin E (see Slater, Benedetto, Burton, Cheeseman, Ingold and Nodes, 1984; Burton, Cheeseman, Ingold and Slater, 1985; Cheeseman, Collins, Proudfoot, Slater, Burton, Webb and Ingold, 1986); ß-carotene (Burton and Ingold, 1984); glutathione peroxidase (see Tappel, 1980); catalase and superoxide dismutase (see Fidovich, 1976).

However, the relative importance of each antioxidant *in vivo* is still not clearly understood, due mainly to the fact that most of these experiments designed to explore biological activity have focused on one known antioxidant. There is evidence to suggest that antioxidants do not function in isolation but act synergistically to produce an overall effect greater than could be provided by a single antioxidant working alone (Barclay, Locke and MacNeil, 1983; see Willson, 1983; see Gey, 1986). Vitamin C for example, can help maintain vitamin E levels (Niki *et al.*, 1982; Barclay *et al.*, 1983; see Willson, 1983). From studies on the peroxidation of methyl esters of linoleic and linolenic acids in the presence or absence of vitamin E and/or vitamin C, it has been shown that the formed lipid molecules react immediately with oxygen to form peroxyradicals; these in turn are scavenged by α-tocopherol to form the α-tocopheroperoxyl radical which is reduced to α-tocopherol by ascorbic acid. An interaction between selenium and vitamin E is well documented (Trinder, Hall and Reton, 1973; Julien, Conrad and Moxon, 1976). Selenium can in some instances substitute for vitamin E deficiency (Muth, Oldfield, Remmert and Schubert, 1958; Sharman, Blaxter and Wilson, 1959). The basis of this lies in the fact that selenium is the metal constituent of glutathione peroxidase, also a major

component of the antioxidant systems (Rotruck, Pope, Ganther, Swanson, Hafeman and Hoekstra, 1973).

9.1.1 Vitamin E, its structure and antioxidant role in the organism.

Of all the naturally occurring antioxidants, it is generally accepted that vitamin E is by far the most prominent. Its presence in the egg is well documented (Sasago, Kobayashi and Tsugo, 1974; McLaughlin and Weihraugh, 1979). Vitamin E, as it occurs naturally, consists of eight compounds which belong to two series of methyl-substituted derivatives of tocol. They comprise a chroman-6-ol nucleus with either a saturated (the tocopherols) or unsaturated (the tocotrienols) side chain in the 2 position (see Schudel, Mayer and Isler, 1972; see Kasparek, 1980; see Parrish, 1980). The tocopherols and tocotrienols are designated alpha (α), beta (ß), gamma (γ) and delta (δ) and differ according to the number of the methyl groups in the chromanol nucleus (Figure 3). Of the clearly related tocopherols, the alpha is by far the most important biologically (Leth and Sφndergaard, 1977), which indicates a high degree of compound specificity with regard to function. The structure and biological activities of the tocopherols and tocotrienols are well documented (see Schudel *et al.* 1972; see Kasparek, 1980; see Parrish, 1980).

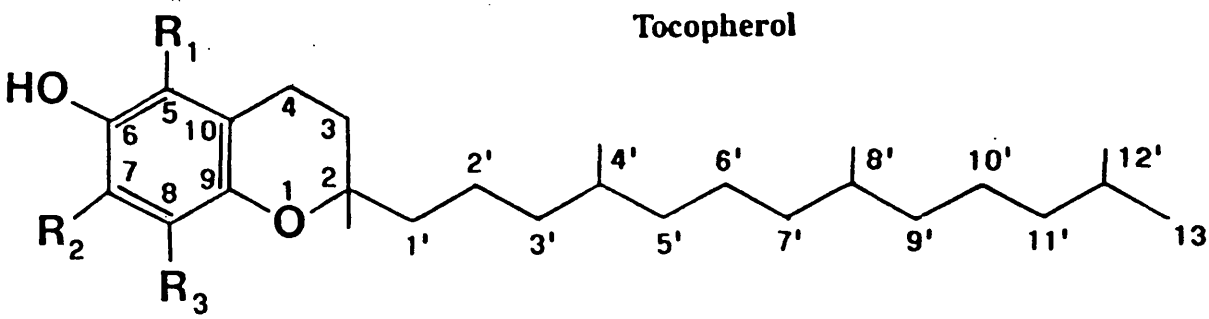
9.1.2 The antioxidant role of vitamin E and its importance in cellular systems.

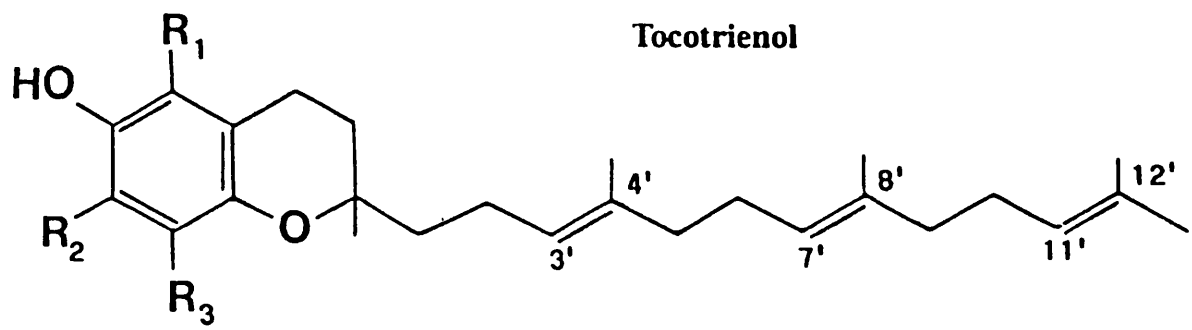
There is evidence to support the role of vitamin E in ensuring membrane stability and associated tissue function. Thus, studies have shown that there was an increased susceptibility of erythrocytes to lysis under restrictive conditions of vitamin E in birds and mammals (György and Rose, 1949; Fisher, Nelson and Young, 1970; Horn, Barker, Reed and Brin, 1974; Siddons and Mills, 1981).

Figure 3

Chemical structures of the E vitamers (Putman and Wagland, 1990).

Figure 3





Tocopherol or Tocotrienol	R_1	R_2	R_3
α-5,7,8-Trimethyl	CH_3	CH_3	CH_3
ß-5,8-Dimethyl	CH_3	H	CH_3
ɣ-7,8-Dimethyl	H	CH_3	CH_3
δ-8-Methyl	H	H	CH_3

Early studies on α-tocopherol deficient chicks noted that fatty acids of the linoleic acid (n-6 series) found in cod liver oil prevented *in vivo* conversion of linoleic acid to arachidonic acid and subsequent alteration to phospholipids in various tissues (Century and Horwitt, 1964). A substantial role for vitamin E in the interconversions of higher polyunsaturated fatty acids was therefore implicated (Horn *et al.*, 1974). Vitamin E prevents free radical accumulation by scavenging (see Tappel, 1975); protects polyunsaturated fatty acids by specific intermolecular association of the tocols with the polyunsaturated fatty acids (Lucy, 1972; Diplock and Lucy, 1973) and functions as a lipotrophe in the removal of polymerised polyunsaturated fatty acids and other unusual lipids (Hayes, Nielson and Rousseau, 1969). Detailed descriptions of these functions at the cellular level have been the subject of several reviews (see Frankel, 1982, 1985).

Recently, a specific role for vitamin E in the interrelationships between C18 and C20 polyunsaturated fatty acids and prostaglandin biosynthesis has been identified (see Sheffy and Williams, 1980; see Tengerdy, Mathias and Nockels, 1981; Panganamala and Cornwell, 1982). Outwith its protective role, vitamin E has also been shown to have a range of other functions which include aspects of protein metabolism (see Putman and Comben, 1987); immune function and development (Tengerdy, Heinzerling, Brown and Mathias, 1973; Jackson, Law and Nockels, 1978; Tengerdy and Brown, 1977; see Tengerdy *et al.*, 1981); reproduction (Trinder *et al.*, 1973; Julien *et al.*, 1976; Kamardin, 1978); the stabilisation of nervous and muscular-skeletal systems (Jackson, Jones and Edwards, 1983; Phoenix, Edwards and Jackson, 1989).

9.1.3 Gross effects of vitamin E deficiency.

In the chick embryo, deficiency of vitamin E is known to manifest itself through a series of conditions. Embryo mortality increases considerably. Under less extreme conditions, there is a notable retardation in growth. The principal causes of death arise from haemorrhaging and a failure of adequate development of the circulatory system (Adamstone, 1931; Singsen, Matterson, Kozeff, Bunnell and Juncherr, 1954; Couch and Ferguson, 1972). With the reliance after hatching on remnant yolk lipid surplus, effects on the emerged chick have also been shown that involved death, slow growth rate, leg weaknesses and other growth abnormalities (Singsen *et al.*, 1954; Kristiansen, 1973; see Scott, 1980). Associated clinical and histopathological changes in various animal species have been noted (Singsen *et al.*, 1954; Irving and Watts, 1962; Kristiansen, 1973; see Scott, 1980; Rice and McMurray, 1982. Bieber-Wlaschny, 1988; see McMurray, 1990).

10 The present studies.

Extensive investigations into the concentration changes of some of the major lipids and their fatty acid compositions of the yolk, yolk sac membrane and tissues of the chick embryo have enabled a range of metabolic interpretations to be made on the development of the tissues. However, with regard to the fundamental aspects of cellular physiology and metabolic regulation, there remain many unanswered questions. For instance, are the interactions between the lipids of membrane bilayers during development of the chick embryo of a sufficient magnitude to modulate the activity of membrane structure and function? The presence of a large accumulation of polyunsaturated fatty acids in the various embryonic tissues during development necessitates the need for antioxidant protection. Thus the relationship between α-tocopherol levels in the fertile egg and subsequent polyunsaturated fatty acid metabolism may be sufficiently tenuous to put normal development at risk. In recent years, a major feature with respect to hatchability and early chick survival, has been observed in the broiler industry. Lowered hatchability and reduced chick survival presents a considerable problem in offspring from young parents. An association with lipid transfer and subsequent incorporation into the embryo has been implicated.

The present study is concerned with an investigation at a fundamental cellular level into the role of lipid metabolism in the control of tissue development and function during the final week of incubation and subsequently the early post-hatching period. Particular reference is paid to three principal tissues in the major aspects of lipid metabolism, namely the liver, heart and brain.

CHAPTER 3
MATERIALS AND METHODS

1 Source of eggs and incubation procedure.

1.1 Eggs.

Fertile eggs were obtained from flocks of Ross 1 broiler-breeder parent stock (Ross Poultry Farms Ltd., Inverurie, Scotland) at twenty-three to twenty-four and thirty-seven to forty weeks of age, young and mature parents respectively. All flocks had been reared on deep litter and received a proprietary diet formulated for breeding birds. The eggs were transported from the farm to the incubator in a suitable vehicle, with maximum emphasis given to their protection during transportation and transfer.

1.2 Incubation.

The incubation conditions for all experiments were identical. However, the number of eggs set varied between experiments.

The eggs were arranged on incubator trays in a standard manner, one hundred eggs per tray and incubated in a forced draught Model TB4 Incubator (Western Incubators Ltd., Essex, England) fitted with fans to maintain an adequate circulation of air. The temperature of the air inside the incubator was controlled by high grade mercury-in-glass thermometers fitted with platinum electrode contacts which were set to a temperature of 37.8°C for the first eighteen days of incubation and 37.5°C for the subsequent hatching period. Automatic turning of the eggs was hourly to maximise hatchability, that is, to promote viability in the early stages of the incubation and to prevent adhesions at the later stages. The eggs were tilted around their short axis and were set in the trays with their narrow end downwards. The angle of turn was sufficient to prevent malformations. The humidity, which was maintained by an

appropriate water tray, was adjusted to give approximately 53 per cent (DB/WB) for the first eighteen days of incubation and then to approximately 48 per cent (DB/WB) just prior to hatching.

2 Environment and rearing of chicks.

By the end of day 21, 89 per cent of the eggs from the mature parents, that is, thirty-seven to forty weeks of age, had fully hatched compared with only 50 per cent from the young parents, that is, twenty-three to twenty-four weeks of age.

Transport of the chicks from the hatchery to the rearing house was by suitable vehicle, taking care not to expose the chicks to any excessive stresses. No post-hatch sexing was done.

The rearing conditions for all short term experiments were initially the same with modifications to the environment being introduced in experiments involving greater than two weeks post-hatch.

There were no differences in the rearing conditions for the chicks derived from the two parental ages. Normal commercial practice was adopted throughout.

The chicks were reared from hatch until around two weeks of age in a four level Eltex Tier Brooder (Elt Ltd., Eltex Works, Worcester, England), each level having a galvanised mesh floor and capable of holding one hundred and fifty day- old chicks. Chick numbers were always kept below maximum stocking density. At around two weeks of age all birds, excess to requirements, were culled to leave two groups, each of twenty birds, which were transferred to single wooden pens and reared on wood shaving litter of about 3cm thick. Precautions against disease and infestations were

made according to recommended procedures (Ross Breeders Ltd., 1985). The birds were kept as single groups, based on parental age, at a stocking density of about $0.2m^2$ per bird.

The birds were reared under a programme of continuous lighting for 23.5 hours at a recommended 20 lux fluorescent source for the first week, gradually decreasing to 2-3 lux thereafter. Initial temperature (day 1) in the Eltex Tier Brooder was 32°C, reduced thereafter, at a rate of 0.5°C per day. The design of the tier brooder facilitated natural ventilation. The overall in-house temperature for the pens was maintained at 24°C$\pm$0.5°C using a draught-free extractor fan connected to a graduated thermostat switch.

Each group received, until three weeks of age, a commercial broiler starter crumb (Dalgety, Glasgow, Scotland), fortified with growth promoters and coccidiostats (Avatec®, Roche products Ltd., Vitamin and Chemical division, Hertfordshire, England). Broiler grower pellets were provided from three weeks until five weeks of age and thereafter, broiler finisher pellets up to slaughter at six weeks of age. Feeding was *ad libitum* via standard egg trays in the tier brooder and circular tube feeders in the pens. Water was unmedicated and freely available at all times from troughs within the tier brooder and standard auto-fonts in the rearing pens. Drinkers and feeders were thoroughly cleansed each day before replenishment.

The litter was turned every three days to prevent dampness. Vaccination by aerosol against infectious bronchitis was performed immediately after hatch (Poulvac® IB). H120, Duphar B.V., Weesp, Holland). A door-step disinfectant was used to reduce the transfer of fomite diseases.

3 Sampling.

Embryonic sampling of eggs from young and mature parents was conducted at days 13, 16 and 19 of incubation (13d, 16d, 19d). In accordance with the Management Manual (Ross Breeders Ltd. 1985), for the mature parents, fertility and chick hatchability rates were well in excess of 90 per cent and 85 per cent respectively, with peak hatchability obtained with parents of thirty-five weeks of age (Figure 4). Fertility rates were also high for the young parents but chick hatchability rates were only in the region of 50 per cent.

At each day of sampling, livers, hearts and brains were discretely excised from the embryos and sufficient material for analysis obtained by requisite pooling which varied with the age of the embryo. The selection of embryos for sampling was entirely random. In all cases the number of embryos used provided a representative sample of the chick group.

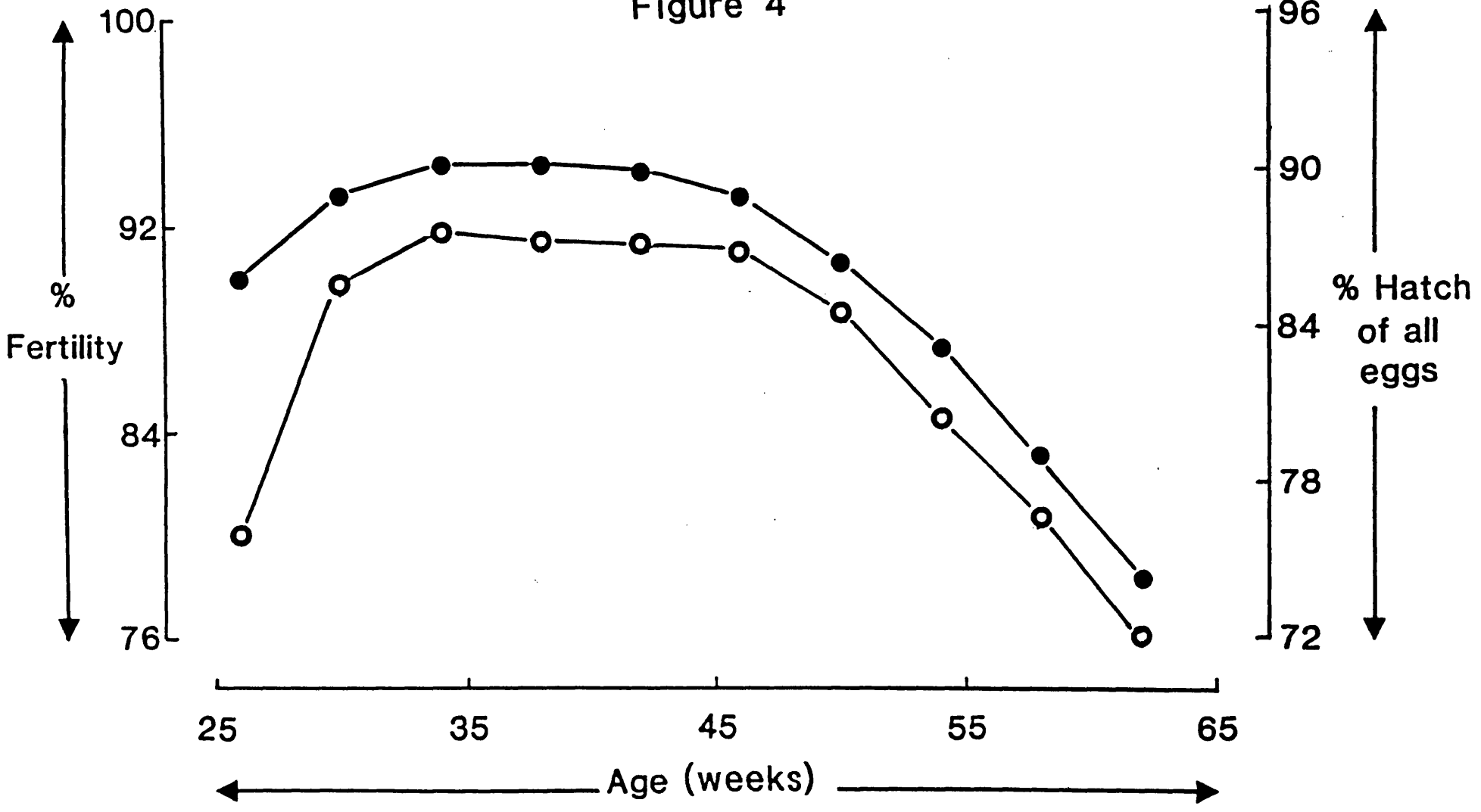
Samples of chick tissues were obtained at days 1, 6 and 12 of post-hatch (1DO, 6DO, 12DO). The chicks were killed humanely and instantaneously by cervical dislocation (see Animal [Scientific Procedures] Act, 1986) and dissected as for the embryos. Although sufficient material for subsequent analysis could be easily derived from small numbers, nevertheless pooling, involving sufficient numbers of chicks, was indulged in to ensure uniform representation of groups. Excess tissue not required for immediate use, was stored in a deep freeze maintained at -20°C. Excision of the tissues was as follows:

The embryos were killed by Schedule 1 Method (see Animals [Scientific Procedures] Act, 1986). After anaesthetisation by injection with nemutal, an incision was made at the blunt pole of the egg and a cut continued along the longitudinal axis of the egg

Figure 4

The standard performance of Ross 1 broiler-breeder parents in terms of their fertility (●) and chick hatchability rates (○). For simplicity, not all the weekly fertility and chick hatchability rates are included in the graph but the overall trend is shown. (Data obtained by kind permission of Ross Breeders Ltd., Broiler Parent Stock Management Manual (1985) No. 208).

Figure 4


100
92
84
76
% Fertility
96
90
84
78
72
% Hatch of all eggs
25
35
45
55
65
Age (weeks)

shell to expose the embryonic membranes and embryo. The embryo, its associated embryonic membranes and yolk sac, were then carefully removed and transferred into a glass petri-dish. Starting from the ventral surface of the embryo, dissection was carefully performed to reveal the liver, heart and brain were carefully removed. Care was taken to prevent puncture of the other organs, particularly the gall bladder. Once excised, each tissue was immediately chilled to 4°C in a beaker containing 0.85 per cent (w/v) sodium chloride solution kept in an ice bucket. Blotting of the tissues on filter paper was kept to a minimum, in view of their fragility.

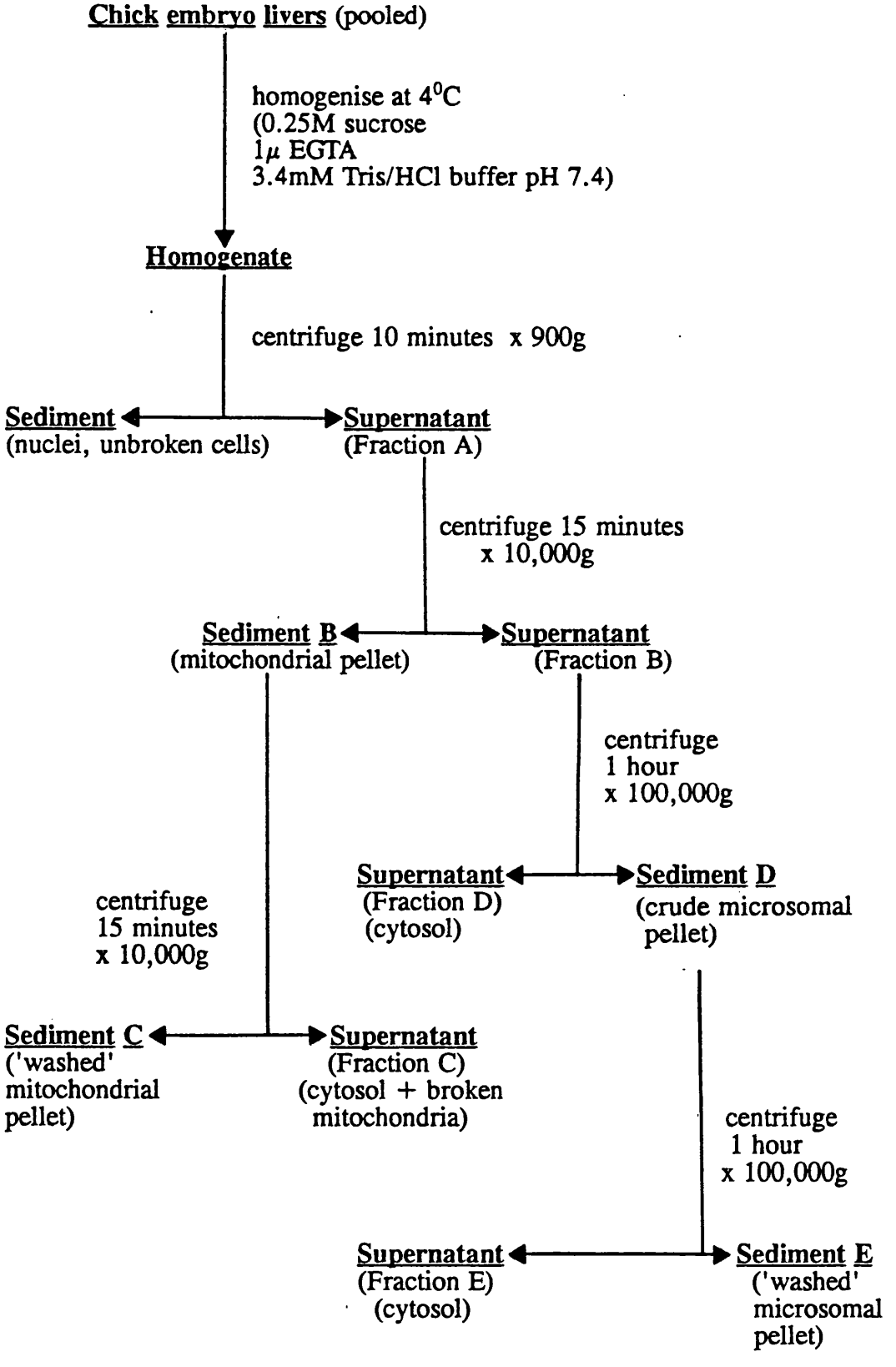
Sampling procedures were identical for the embryos and chicks from the two parental age groups.

4 Isolation of the subcellular tissue fractions.

The general procedure, as applied to the liver, is diagrammatically presented in Figure 5. The livers were removed from the saline solution and gently blotted with tissue paper. Following weighing on a two decimal place balance (Mettler PL1200, Mettler Instruments, Zurich, Germany), the livers were transferred into a fresh solution of cold constantly mixed buffer medium (0.25M sucrose and 1μM ethyleneglycol-bis-[ß-amino-ethyl ether] N, N'-tetra acetic acid [EGTA] in 3.4mM trizma base [Tris] / 1 M hydrochloric acid buffer, adjusted to pH 7.4 at 4°C). Homogenisation to break down the tissue was then manually performed in a 55ml borosilicate glass Uni-form homogeniser with five passes of a loosely-fitting PTFE pestle in the presence of the buffer. The ratio of buffer to liver tissue was maintained at 1 to 5 (W/V). The pestle diameter was less than 25mm with a gap of more than 0.126mm all round clearance between it and the homogenisation tube. All accretions of connective tissue elements were removed by subsequent homogenisation using a tight fitting PTFE pestle, 25mm in diameter, giving a gap of 0.076mm to 0.126mm all round clearance.

Figure 5

Flowsheet for the fractionation and isolation of the mitochondria and microsomes of embryo and neonatal chick liver.


Chick embryo livers (pooled)
homogenise at 4°C
(0.25M sucrose
1μ EGTA
3.4mM Tris/HCl buffer pH 7.4)
Homogenate
centrifuge 10 minutes x 900g
Sediment
(nuclei, unbroken cells)
Supernatant
(Fraction A)
centrifuge 15 minutes
x 10,000g
Sediment B
(mitochondrial pellet)
Supernatant
(Fraction B)
centrifuge
1 hour
x 100,000g
Supernatant
(Fraction D)
(cytosol)
Sediment D
(crude microsomal
pellet)
centrifuge
15 minutes
x 10,000g
Sediment C
('washed'
mitochondrial
pellet)
Supernatant
(Fraction C)
(cytosol + broken
mitochondria)
centrifuge
1 hour
x 100,000g
Supernatant
(Fraction E)
(cytosol)
Sediment E
('washed'
microsomal
pellet)

Aliquots of the homogenate were then quantitatively transferred to four standard 55ml flanged polypropylene centrifuge tubes (internal diameter, 9mm; length, 10.2cm) and their weights accurately balanced using buffer medium. The samples were centrifuged for 10 minutes in an 8x50ml SS-34 aluminium, angle head rotor in a Sorvall RC-5B refrigerated centrifuge (Du Pont Company, Delaware, U.S.A.) at an average speed of 3000rpm, delivering a relative centrifugal force of 900*g*. The rotor speed chosen was related to the relative centrifugal force in an uncapped, partially-filled tube with a minimum to average allowable volume of sample (MSE Technical Publication, 1981).

The pellets resulting from the centrifugation contained the red blood cells, residual intact liver cells and free nuclear debris and were thus discarded. The amount of superficial fat within the supernatant varied extensively depending on tissue, age etc. and was carefully removed with a spatula, retained and analysed separately. The fat layer however, did not form part of the overall quantification procedure between the subcellular fractions. The tubes were then reweighed, rebalanced by the addition of buffer (Fraction A) and centrifuged for 15 minutes at 9500rpm, delivering a relative centrifugal force of 10,000*g*. This yielded the crude mitochondrial pellet. The supernatant (Fraction B) was retained for further centrifugation.

The firmly packed, opaque sediment of crude mitochondria was resuspended in 15ml of buffer solution, the tubes weighed and rebalanced. In order to effect purification and to free the mitochondrial preparation from the soluble substances arising from contamination by the original homogenate, the mitochondria were resedimented at a gravitational force of 10,000*g* (9500 rpm). The supernatant (Fraction C) was retained and the pellet washed and resuspended in 5ml of buffer. Preparations of liver samples from embryos at days 13, 16 and 19 of incubation, and chicks at days 1, 6 and 12 were washed a further two times to ensure maximum removal of any

superficial fat contamination. The final mitochondrial pellet was pale brown in colour and exhibited the obvious birefringence of flow when stirred.

Mitochondria of the brain and heart were isolated in an analogous manner as that depicted above for the liver but, in the case of the heart tissue, the method of homogenisation was modified slightly to take into account the presence of extensive connective tissue. The hearts were chopped into very fine pieces (cubes no greater than $1mm^3$ in size) and homogenised in the Uni-form homogeniser as described above. The resultant homogenate was then divided further, homogenised in a 30ml borosilicate glass uni-form homogeniser fitted with a tightly-fitting PTFE pestle 19mm in diameter, that gave a gap of 0.076mm to 0.126mm. The resulting homogenate was then freed of obvious clumps of connective tissue by transfer back to the normal homogeniser fitting. The fractions derived from both brain and heart tissues were again tested for birefringence.

The method of isolation of the microsomes was the same for all tissues. The post mitochondrial supernatant (Fraction B) was quantitatively transferred to four 25ml polycarbonate centrifuge tubes and accurately balanced by suitable addition of buffer medium. The supernatants were submitted to centrifugation (Prepspin 50, MSE. Scientific Instruments, Sussex, England) for 1 hour at 100,000*g* (36,000rpm) in an 8 x 25ml aluminium angle head rotor. The rotor speed chosen was related to the relative centrigugal force of a capped tube, with an average to maximum allowable volume of sample (MSE Technical Publication, 1981).

The supernatant (Fraction D) obtained following centrifugation was decanted from the crude microsomal pellet and retained for further analysis. The microsomal pellet was resuspended in a suitable quantity of buffer and the microsomes resedimented by a

further centrifugation for 1 hour at 100,000*g*. The suspensions were opalescent and showed birefringence of flow on stirring. Supernatants, with respect to the liver, were represented by material following removal of the fat layer. In the case of the heart and brain, a similar fat layer did not exist.

5 Lipid analysis.

5.1 Extraction.

Extraction of the total lipid was by homogenisation in a suitable quantity of chloroform: methanol (2:1 v/v) according to the standard procedure of Folch, Lees and Sloane-Stanley (1957). Following the addition of a suitable volume of methanol and shaking, an equal volume of chloroform was added and the mixture refluxed for 20 minutes at 60°C. Following cooling, a further aliquot of chloroform was added, the mixture shaken and filtered into a measuring cylinder.

To the total filtrate was added one fifth by volume of a 0.88 per cent (w/v) solution of potassium chloride. The mixture was shaken and allowed to partition into two layers over a period of 2 hours. The upper aqueous layer containing dissolved aqueous-soluble contaminants was removed by aspiration and discarded. The lower organic phase, containing the extracted lipids, was evaporated to dryness on a rotary film evaporator at 40°C. The complete removal of water was ensured by sequential evaporations. The lipid was then taken up in 5ml of chloroform, transferred to suitable glass vials and stored in a refrigerator at 4°C for subsequent analysis.

5.2 Separation.

The major lipid fractions were separated as discrete bands by thin layer chromatography on absorbent layers, 0.25mm in thickness, of silica gel G (E, Merck, A-G, Darmstadt, Germany), by development in a solvent system of hexane: ether: formic acid (80:20:1 v/v/v). The plates were prepared manually by standard procedure (see Christie, 1982) using a mixture of 22.5g of Kieselgel G in 50ml of distilled water and a commercial spreader. Following drying in air for 30 minutes, the thin layer plates were activated in an oven at 120°C for 1 hour and stored in a suitable sealed box in the presence of dehydrated 'self-indicating' silica gel crystals. The plates were reactivated in the oven for 1 hour before use. The developing solvent mixture was freshly prepared in all cases and the effective solvent humidity within the developing tank was maintained by lining with filter paper.

Suitable aliquots of lipid extracts were applied as discrete bands, approximately 2cm from the edge and 2cm from the base of the thin layer plate. Following a brief period of drying, the plate was then developed.

Separation of the major lipid classes was deemed to be complete once the solvent front was approximately 3cm from the top of the plate. The plate was briefly air dried to remove excess solvent and then lightly sprayed with a 0.1 per cent (v/v) solution of 2-4-dichlorofluorescein in methanol. The separated lipid bands were identified under ultra violet light (366 nm) by comparison with known standards. Quantitative visual records where required, were obtained by Polaroid photography following charring (see Section 5.4). The identified lipid bands were scraped from the plate and the lipids quantitatively recovered from the silica gel by elution with an appropriate solvent by a sequential process of mixing, centrifugation and decantation. The neutral

lipids, that is, cholesteryl esters, triglycerides, free fatty acids, free cholesterol and partial glycerides were eluted from the silica gel by 3 x 5 ml washes with diethyl ether and the phospholipids, by 3 x 5 ml washes with methanol. The eluants containing the lipid fractions were stored in chloroform for subsequent analysis.

5.3 Fatty acid preparation (preparation for Gas Liquid Chromatography).

Following the addition of a suitable amount of a pentadecaenoic acid standard (Christie, Noble and Moore, 1970) to the lipid extract, the whole mixture was evaporated to dryness on a rotary film evaporator and fatty acids converted to their methyl esters by the addition of 4ml of an anhydrous mixture of toluene: methanol: sulphuric acid (10:20:10 v/v/v) and refluxing for 1 hour at 60° C.

Following cooling, the fatty acid methyl esters were extracted by partitioning between equal volumes of hexane and distilled water. The upper hexane layer containing the fatty acid methyl esters was carefully removed and dried by the addition of a suitable amount of sodium sulphate: sodium hydrogen carbonate (4:1 w/w). After a minimum time period of 30 minutes, the hexane layer was decanted off, evaporated to dryness under a stream of nitrogen and then redissolved in a suitable, small volume of hexane for injection onto a gas liquid chromatograph.

Gas liquid chromatographic analyses of the fatty acids were performed using either a Model 428 instrument (Canberra- Packard Instruments Ltd., Berkshire, England) or a Model 4500 instrument (Pye Unicam Phillips Ltd., Cambridge, England). Separation of the methyl esters was achieved on 1.5m to 2.0m packed glass columns, (internal diameter, 2mm and 4mm respectively; outside diameter, 6mm) of 15 per cent CP-Sil 84/Chromosorb WHP 100 to 120 mesh (Chrompack Ltd., Middleburg,

The Netherlands). Nitrogen was used as a carrier gas at a flow rate of 40ml per minute and an oven temperature of between 181°C and 198°C.

Known methyl ester standards were used to check the quantification procedures employed and the identification of the fatty acids. In all cases 1 to 2 μl of sample was injected. Identification of the fatty acids was from their retention times. Unknown peaks were identified using logarithmetic plots of the retention times of known fatty acids. The relative proportions of the fatty acids were quantified by integration of the amplifier signal using an electronic integrator (Spectra Physics Analytical Ltd., Model 4270, San Jose, California). Reference to the pentadecaenoic acid standard peak enabled absolute amounts of the fatty acids to be determined (Christie *et al.*, 1970, Table 4).

5.4 Cholesterol determination.

Quantification of free cholesterol was performed by a densitometric method using a liquid scintillation counter (Shand and Noble, 1980). Standard amounts of cholesterol and samples contained in suitable small volumes of chloroform were applied as uniform 8cm bands onto standard thin layer plates of silica gel. In all case of samples, the free cholesterol was separated by elution (see Section 5.2). The standards and separated free cholesterol were then charred by spraying with an aqueous solution of 3 per cent (W/V) cupric acetate in 8 per cent (W/V) phosphoric acid (Fewster, Burns and Mead, 1969) until translucent and heating at 180°C for 15 minutes in a forced draft oven (Townson and Mercer, Model 8-300, Surrey, England). The charred cholesterol bands were then scraped from the plate and resuspended in a mixture of water (2ml) and emulsifier-scintillator cocktail (10ml) (Fisons plc., Loughborough, England).

Lipid class	Factor
Cholesteryl esters	2.246
Triglycerides	0.995
Free Fatty acids	0.951
Phospholipids*	1.371

* As an approximation, it is assumed that the phospholipids consist of phosphatidylcholine only.

Table 4 The correction factors used to covert the total amount of fatty acids in a lipid class (determined by gas chromatography analysis) to weight of lipid (Christie *et al.*, 1970).

Following vigorous shaking to form a uniform opalescent gel of the charred lipid material, the amount of lipid was determined by measuring the extent of ' quenching ' of secondary beta emissions produced by exposure to an external standard radium^{226} source in a liquid scintillation counter (LKB Wallac, Model 1215, Turkun, Finland). By reference to a standard curve relating the amount of free cholesterol to the change in the external standard channel of the radium^{226} source, the absolute amounts of free cholesterol present in the sample could be determined.

5.5 Determination of desmosterol/cholesterol.

Determination of desmosterol/cholesterol was performed by Gas Liquid chromatography using betulin as an internal standard. The sterols were transmethylated according to a standard procedure (see Document VI/14275/87) produced by the Commission of the European Community (1987). To the dried sample (approximately 2mg) was added 1ml of a mixture of pyridine (Silylation grade): hexamethyldisalazine: trimethylchlorosilane (9:3:1 v/v), the vial capped and shaken vigorously for 30 seconds. The reaction vial was left to stand at room temperature for 40 minutes to allow complete derivatisation.

Gas Liquid chromatography of the sterol derivatives was performed using a Model 437A instrument (Canberra-Packard Instruments Ltd., Berkshire, England) and autosampler. Separation of the cholesterol and desmosterol was achieved on a 1.5m packed glass column (internal diameter 4mm; outside diameter, 6mm) of 3 per cent OV17/ Chromosorb WHP 80-100 mesh (Chrompack Ltd., Middleburg, The Netherlands) at a temperature of 270°C, using nitrogen as the carrier gas at a flow rate of 15ml per minute. The injection volume was 1μl. Identification of the sterols was by comparison of retention times with known standards. Their relative proportions were quantified using an electronic integrator (Spectra Physics Model

4270). The duration of each analysis was such to ensure complete removal of other sterols before commencement of the next sequence.

6 Transmission electron microscopy.

Incubation times were so adjusted as to ensure a coincidence of embryonic and chick sampling times between parental age groups. Following excision of the tissues, transmission electron microscopy investigations of each sample were performed by standard procedures (Gray, Angus and Snodgrass, 1980) under the auspices of the Electron Microscopy Unit of the Moredun Institute in Edinburgh.

Chopped samples were fixed for a minimum of 3 hours in 3 per cent glutaraldehyde in 0.1M phosphate buffer (pH 7.2). All subsequent procedures prior to initial embedding, were performed in a Lynx Tissue Processor (Leica UK. Ltd., Milton Keynes, England). Following suitable washing with buffer, the samples were stored overnight in 0.1M phosphate buffered saline containing 2 per cent dextrose and post-fixed the next day in 1 per cent osmium tetroxide in 0.1M phosphate buffer for 1 hour. After washing three times in 0.1M phosphate buffered saline containing 2 per cent dextrose, the samples were dehydrated in an ascending series of alcohol solutions. Subsequent treatment in two independent solutions of epoxypropane, ensured the removal of traces of ethanol. The dehydrated tissues were stored overnight in epoxypropane and araldite. Initial embedding in araldite took 8 hours at 60°C, followed by final embedding in fresh araldite for 1 to 2 days.

Ultrathin sections (approximately 60-90 nm thick) were cut using a Reichart 'Ultracut' ultramicrotome, fitted with a diamond knife (Leica UK Ltd., Milton Keynes, England). The sections were collected on copper mesh grids (Agar Scientific Ltd., Essex, England), contrasted and stained with saturated aqueous uranyl acetate (Agar

Scientific Ltd.) and 'Reynolds' lead citrate (Reynolds, 1963) and viewed under a Jeol 1200Ex Transmission electron microscope (Jeol UK. Ltd., Hertfordshire, England).

7 Determination of the alpha tocopherol content of various tissues.

In most instances, sufficient material for analysis was provided from individual tissue. However, when sufficient material was not available, pooling was indulged in. The analytical procedure adopted was the same for all tissues but with the addition of an extra washing stage for the yolk sac membrane.

The method used was based on a modification of a standard procedure (McMurray, Blanchflower and Rice, 1980) to take into account the high lipid contents of the samples. To each sample of tissue was added 2ml of 20 per cent (w/v) *L*-ascorbic acid and 8ml of ethanol, the contents shaken and allowed to equilibrate at 70 °C.

To the equilibrated mixture was added 3ml of 60 per cent (w/v) potassium hydroxide in a waterbath for 30 minutes. After cooling, 5ml of water was added, followed by 10ml of hexane: diethylether (60:40 v/v). The solution was mixed vigorously and the layers allowed to separate. The upper organic layer was transferred to a tube containing 10ml of hydrochloric acid (10 per cent v/v), the mixture shaken thoroughly and the layers allowed to separate. After 5 minutes, 5ml of the solvent layer was removed and evaporated off under a stream of nitrogen, 1ml of methanol and 50μl of BHT (2,6-Di-*tert*-butyl-*p*-cresol; 1.2 per cent dissolved in HPLC grade methanol) were added and the solution transferred to an autosampler vial.

A stock solution of a known weight of *dl*-α-tocopherol (21.5mg) was dissolved in 100 ml of ethanol and stored at 5°C. Working standards were prepared daily to give concentrations of approximately 18, 44 and 88μg/g *dl*-α-tocopherol. An 1ml of each

standard was transferred to an autosampler vial and 50μl of BHT was added. Secondary external reference standards of -tocopherol, wheat bran and pastoral barley were also used to assist peak identification. Three independent determinations were made on each batch of samples together with appropriate standards. A multilevel calibration curve was produced from which quantification of the α-tocopherol was possible.

The HPLC (High Peformance Liquid Cromatography) solvent system consisted of a Spectra-Physics SP8700 Solvent delivery system (Spectra Physics Analytical Ltd., San Jose, California), a Varian 9090 autosampler with a Rheodyne injector (Varian Associates Ltd., Cheshire, England) and a Kratos Spectroflow 980 Fluorescence Detector (Applied Biosystems Ltd., Cheshire, England). Quantification was by excitation and detection at 210 and 320nm respectively; the rise time of the detector was 5 seconds. The peaks were quantified by electronic integration using a Spectra-Physics SP4270 Integrator. The data were processed by an IBM PC AT computer using a batch recalculation program (Spectra Physics Autolab Software, Cronstation AT, San Jose, California).

The eluant was methanol: water (97:3 v/v), degassed by helium sparging. The flow rate of the solvent was 2ml per minute at a pressure of approximately 2100 psi. The injection volume was 20μl. Separations were performed at ambient temperature using a 30cm x 4.6mm, 10μm Spherisorb C18 column. Separation of the α-tocopherol γ-tocopherol and tocotrienol peaks was found to be complete in 8 minutes (Figure 6).

Before commencement of sample analysis, the system was calibrated by an injection of 20μl from each of the three working standards. The signal was evaluated by means of peak area integration. Standard calibration was performed prior to each sample run.

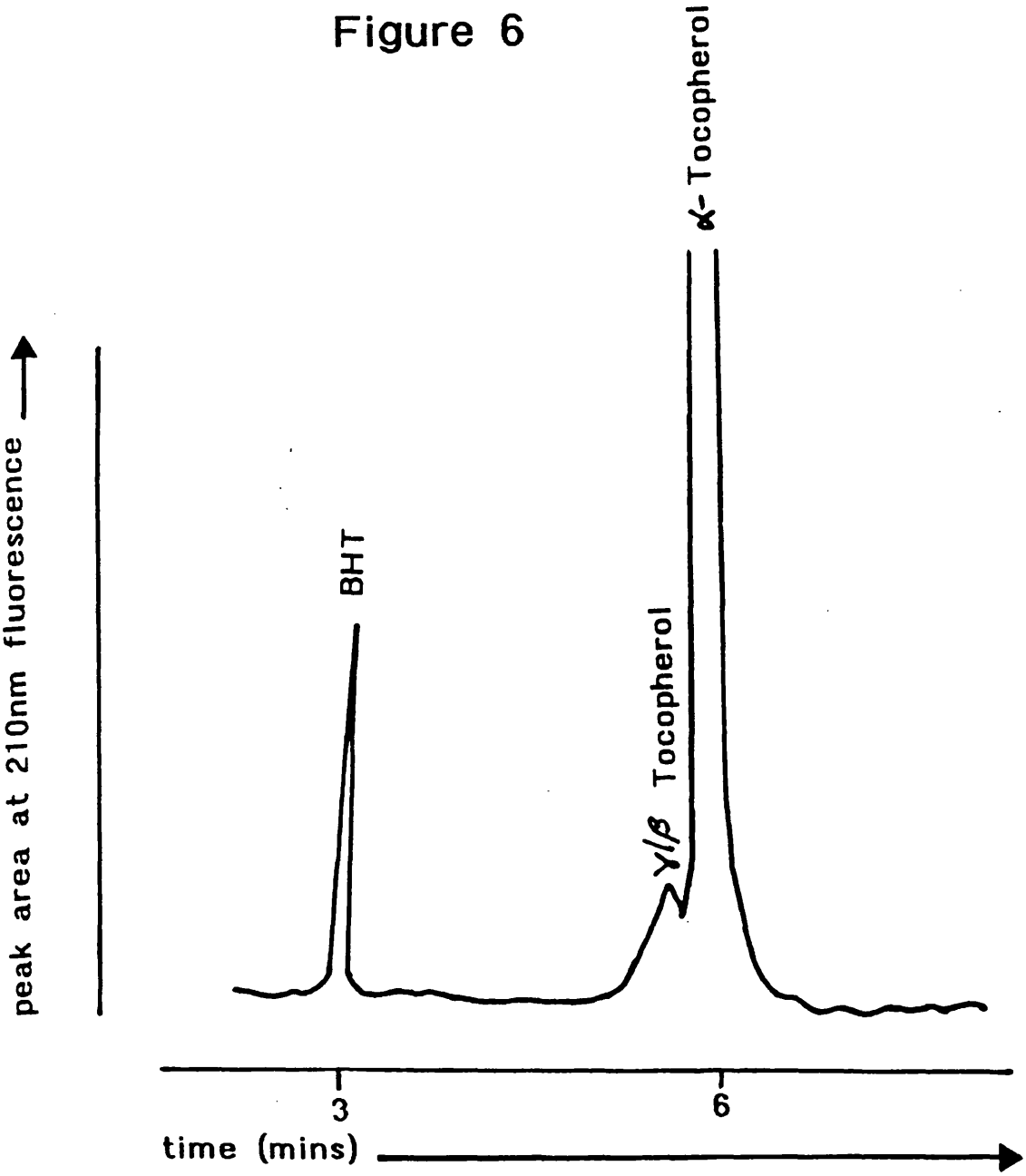
Figure 6

Separation of alpha and gamma tocopherols

using reversed phase high liquid chromatography.

Note: on this type of column beta and gamma tocopherols are coeluted.

Figure 6


peak area at 210nm fluorescence
BHT
γ/β Tocopherol
α- Tocopherol
3
6
time (mins)

8 Statistical evaluation.

Significance of differences in all experiments were investigated by the Student's *t*-test. In the case of α-tocopherol analysis by HPLC, multilevel calibration frequently produced curves with origins which were not zero (Figure 7). To avoid bias in the results at extreme ends of the scale, the multilevel calibration curve was studied for each analysis and where necessary, the results recalculated for a single point using a batch recalculation program (Spectra Physics Autolab Software) on the computer.

9 Materials, solvents and chemicals.

All gas liquid chromatographic packing material and ancillary items were obtained from Chrompack UK., Ltd., Middleburg, The Netherlands. All solvents were of the highest grade possible (e.g. BDH Chemicals Ltd., Dorset, England; Hayman Ltd., Essex, England). Scintillation chemicals and ancillary items were obtained from Fisons plc. Loughborough, England and Canberra-Packard Ltd., Pangbourne, England. All other chemicals and reagents were of analytical grade and obtained from reputable sources. In all lipid analytical investigations, necessary precautions were taken to keep chemical breakdown to a minimum.

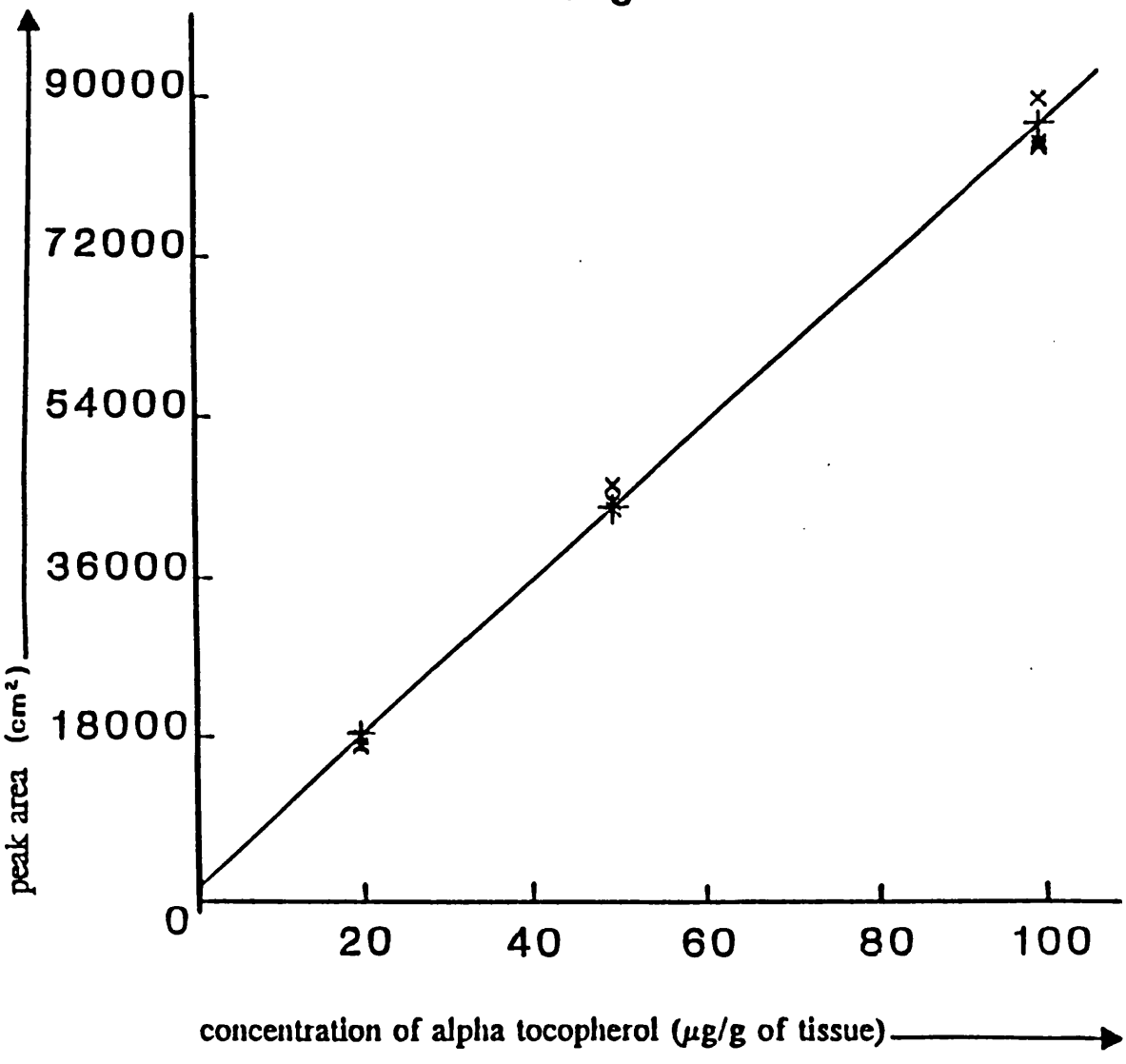
With the exception of propylene oxide, all the chemicals and materials used throughout the electron microscopic analysis were of the required grade (Agar Scientific Ltd., Essex and Hayman Ltd., Essex, England).

Figure 7

Calibration curve for the determination of alpha tocopherol

(Vitamin E in micrograms per gram of wet tissue).

Figure 7


90000
72000
54000
36000
18000
0
peak area (cm²)
20
40
60
80
100
concentration of alpha tocopherol (μg/g of tissue)

CHAPTER 4
RESULTS

Tables for the results section can be found in Chapter 7 (Volume 2). Figures and plates are distributed accordingly throughout the text in Chapter 4.

1 Overall lipid metabolism.

An initial study was carried out to indicate the increasing importance of lipid uptake during the last week of normal embryonic development and during the immediate post-hatch period. The reduction that occurs in lipid uptake in progeny from young parent stock was also analysed and comparative studies in the liver were chosen as a marker of these developmental features.

Table 5 shows the total amount of lipid associated with the liver during the last week of embryonic development and the early post-hatch period. Plates 5a, b, c, d,e,f are electron micrographs of the livers obtained from embryos and chicks from mature and young parents. As can be seen (Table 5), the absolute amount of lipid associated with the liver in embryos from mature parents increased dramatically over the last week of incubation; levels increasing from 18.7mg at day 13 of incubation to 99.8mg at hatching. Although embryos from young parents also displayed an increase, accumulations were significantly less (Table 5). Following hatching, absolute amounts of lipid associated with the liver underwent further large increases (Table 5). Confirmation of the changes in lipid concentrations during the embryonic and post-hatch periods can be seen from the respective electron micrographs (Plates 5a,b,c,d).

Although lipid accumulation was discernible within the cytoplasm of embryos from mature parents at day 13 of incubation (Plate 5a), it can be seen that at day 19, the amount of accumulated lipid had become considerable and was present as large droplets, that occupied the bulk of the cytoplasm of the liver cells (Plate 5b).

Plate 5a

Electron micrograph of chick embryo liver cells at day 13 of incubation. The results are for embryos from mature parents.

Plate 5b

Electron micrograph of chick embryo liver cells at day 19 of incubation. The results are for embryos from mature parents.

Plate 5c

Electron micrograph of chick embryo liver cells at day 13 of incubation. The results are for embryos from young parents.

Plate 5d

Electron micrograph of chick embryo liver cells at day 19 of incubation. The results are for embryos from young parents.

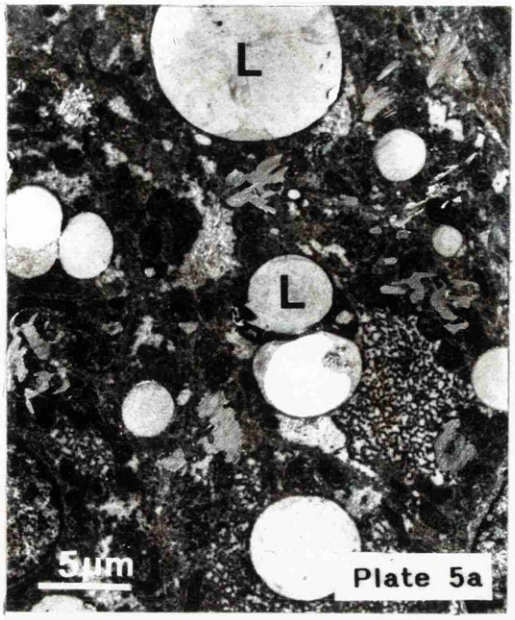
Plate 5e

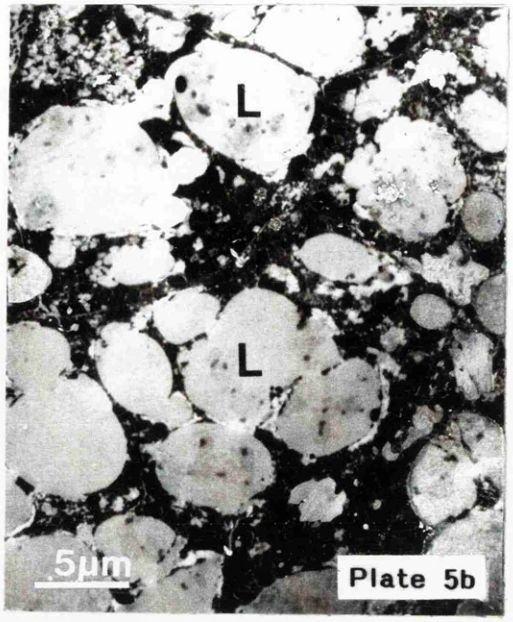
Electron micrograph of liver mitochondria at day 13 of incubation. The results are for embryos from mature parents

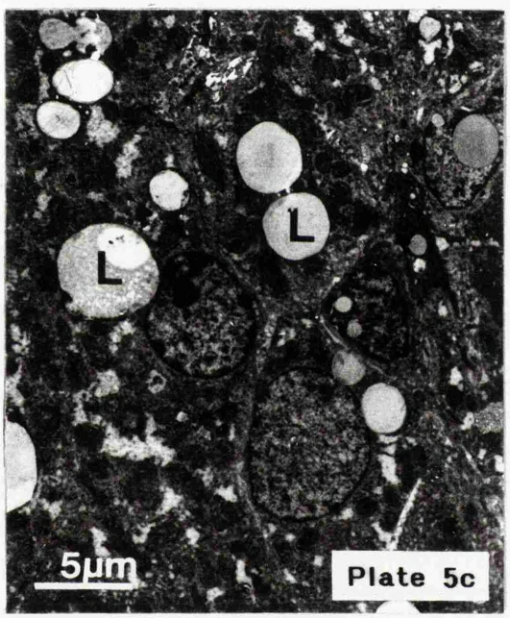
Plate 5f

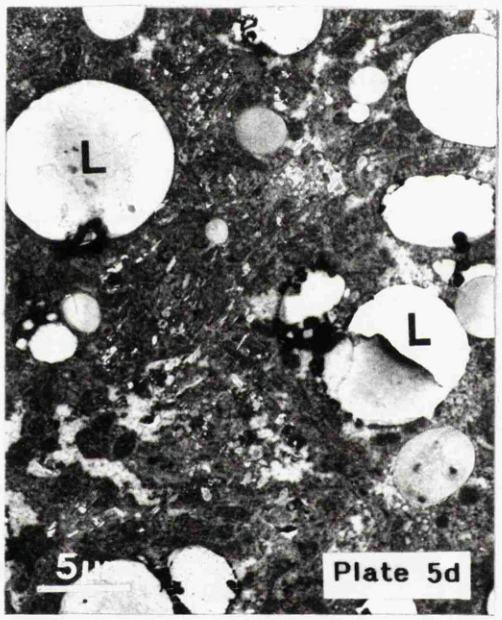
Electron micrograph of liver mitochondria at day 6 of post-hatch. The results are for offspring from mature parents.

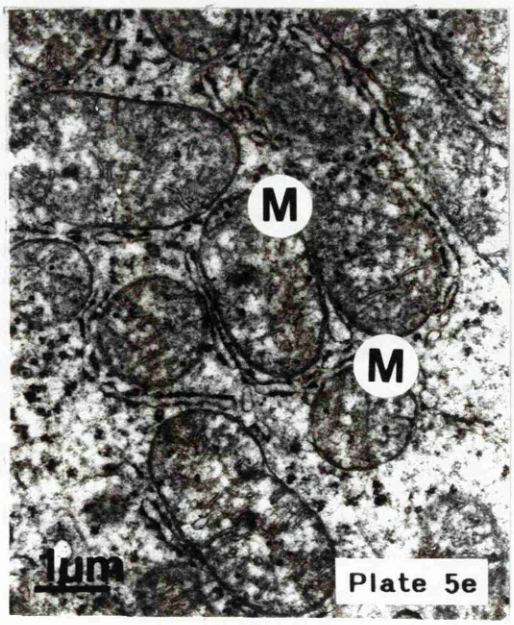
L = lipid droplets
M = mitochondria

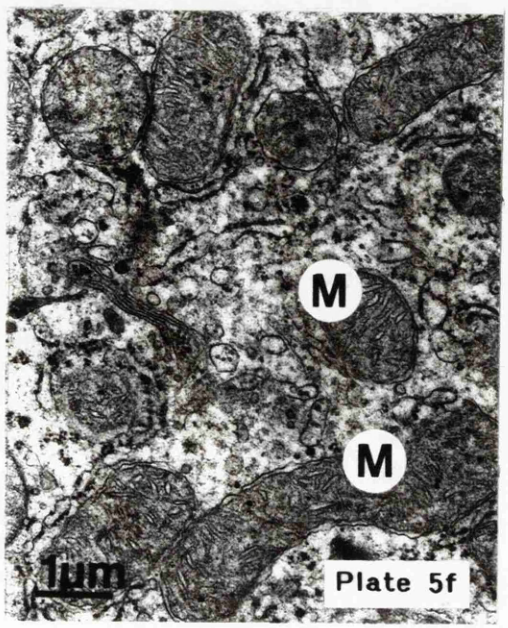

L
L
5µm
Plate 5a


L
L
5µm
Plate 5b


L
L
5µm
Plate 5c


L
L
Plate 5d


M
M
1µm
Plate 5e


M
M
1µm
Plate 5f

Contrastingly, in support of the absolute quantitative lipid measurements, the liver cells of embryos from the young parents at day 19 showed very much less cytoplasmic lipid accumulation (Plate 5d). Following hatching there was a decrease in the number of lipid droplets present in the liver cytoplasm (plates not shown in the text). Throughout both the incubation and post-hatch periods, the mitochondria showed a considerable pattern of pleomorphism (Plates 5e, f), the extent of which was no greater than that expected of liver tissue in general.

2 General appearance of isolates.

The following remarks refer to the visual appearance of the isolates immediately after final centrifugation. Whereas the mitochondria from both the liver and heart appeared pale brown in colour, the microsomes were distinctly red. By comparison with the liver and heart, the mitochondria of the brain were much paler in colour, whilst the microsomes had a creamy white appearance. The membrane suspensions from all three tissues were opalescent and showed birefringence on stirring.

Depending on the amount of lipid present, the liver supernatant varied from colourless and almost transparent (Fraction C) to bright yellow and opaque (Fraction D). The colour of the supernatant was most apparent in isolates obtained during the separation of the microsomes. The heart supernatant varied from colourless to a translucent red. As in the case of the liver, the colour intensity was more obvious in the isolate obtained during the separation of the microsomes. The brain supernatants were colourless and varied in opacity.

3 Major lipid fractions.

Plate 6 shows a typical thin layer chromatographic separation of the major lipid constituents of a subcellular lipid extract. As can be seen, the major lipid fractions in all instances were cholesteryl esters, triglycerides, free cholesterol and phospholipids. Their respective retention factors (RF values) were similar to those recorded previously in the literature. A feint narrow band identifiable as free fatty acids was observed in all the separations. Although their concentration varied, the proportion of the free fatty acids never exceeded 5 per cent of the lipids extracted from the mitochondria and microsomes; other than in a few cases, the concentration of free fatty acids in the lipid of the supernatant fractions was similarly low. Although bands of mono and diglycerides were sometimes identifiable, their presence in the lipid extract was variable.

3 Concentrations of total lipid in the subcellular fractions.

3.1 Liver.

The total concentrations of lipid per gram of tissue, associated with the mitochondria, microsomes and supernatant of the livers of embryos and chicks from mature and young parents, are given in Table 6. The statistical significances of changes in the lipid concentrations of the fractions with increasing embryonic and post-hatch age and the differences between the embryos and chicks from mature and young parents are given in Tables 7a b.

Overall, the mitochondria and microsomes showed similar lipid concentrations to each other during the last week of incubation and early post-hatch period, whilst the concentrations of lipid associated with the supernatant fractions were very much lower

Plate 6

A typical thin layer chromatographic separation of the major lipid constituents of a subcellular extract.

CE = Cholesteryl esters

TG = Triglycerides

FFA= Free Fatty Acids

FC = Free Cholesterol

PG = Mono and Diglycerides

PL = Phospholipids

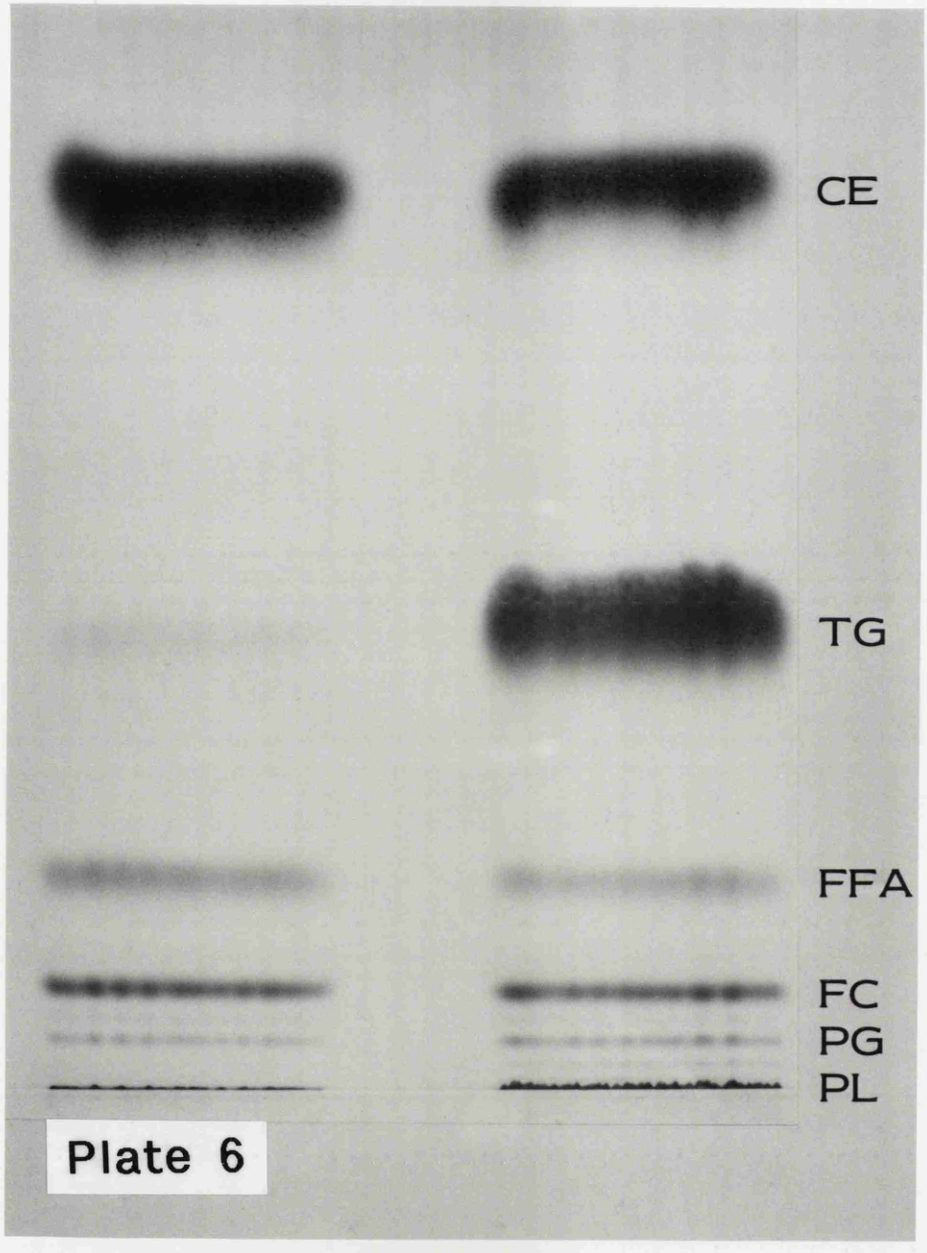


Plate 6

than the values for the mitochondria and microsomes. In the embryos from the mature parents, the concentrations of lipid associated with the mitochondria and microsomes increased between days 13 and 19 of incubation but although the level of lipid in the supernatant more than doubled between days 13 and 16 of incubation, by day 19, its level had been reduced. Moreover, whilst the concentration of lipid associated with the microsomes underwent a decrease during the post-hatch period, concentrations within the mitochondria and supernatants remained largely similar to those observed just prior to hatching.

In the livers of embryos and chicks from young parents, the mitochondria showed much higher levels of lipid in comparison with embryos and chicks from mature parents. Thus, whereas at day 19 of incubation the total lipid associated with the mitochondria of embryos from mature parents accounted for only 2 per cent of the total liver concentration, in embryos from young parents, the value was 4 per cent. Concentrations of lipid within the supernatants and to a large extent the microsomes also, were similar to those for the embryos and chicks from mature parents.

3.2 Heart.

The total concentrations of lipid per gram of tissue, associated with the mitochondria, microsomes and supernatant of the hearts of embryos and chicks from mature and young parents, are given in Table 8. The statistical significances of changes in the lipid concentrations of the major subcellular fractions with increasing embryonic and post-hatch age and the differences between the embryos and chicks from mature and young parents are given in Tables 9a b.

In general, the levels of lipid within the mitochondria and microsomes were similar to each other throughout the embryonic and post-hatch periods. Once again, the concentrations of lipid associated with the supernatant fractions were lower than the values for the mitochondria and microsomes but the differences were less obvious compared with isolates obtained from the liver and the brain. Heart mitochondria from embryos and chicks of mature parents showed a generally consistent level of lipid throughout the final week of incubation and post-hatch period but there was an obvious decrease in the level of lipid in the microsomes during post-hatch.

Embryos and chicks from young parents displayed similar levels of lipid within the supernatant to embryos and chicks from mature parents. However, mitochondria from embryos and chicks from young parents displayed higher levels of lipid during both periods whereas, the microsomes showed higher levels of lipid at post-hatch only.

3.3 Brain.

The total concentrations of lipid per gram of tissue, associated with the mitochondria, microsomes and supernatant of the brains of embryos and chicks from mature and young parents, are given in Table 10. The statistical significances of changes in the lipid concentrations of the fractions with increasing embryonic and post-hatch age and the differences between the embryos and chicks from mature and young parents are given in Tables 11a b.

In overall terms, the levels of lipid within the supernatant fractions, throughout the embryonic and post-hatch periods, were again much lower than levels within the mitochondria and microsomes. Apart from a significant decrease at hatching, brain mitochondria from offspring of mature parents showed a generally consistent level of lipid throughout the incubation and post-hatch periods, whilst the level of lipid

associated with the microsomes showed a decrease during post-hatch. There were no major differences in the levels of lipid within the microsomes and supernatant between the embryos and chicks from mature and young parents. However, mitochondria of embryos and chicks from young parents displayed higher levels of lipid during both periods.

4 The distribution of the total lipid between the mitochondria, microsomes and cellular supernatant of the liver, heart and brain.

The distribution of total lipid between the three subcellular fractions in liver, heart and brain are given in Tables 12, 13 and 14 respectively.

4.1 Liver.

As can be seen, there was a relatively even distribution of total lipid between the three subcellular fractions of the liver at day 13 of incubation; at later stages of incubation, the lipid became more associated with the particulate fractions. During the post-hatch period there was a prominent association of lipid with the particulate components, but especially the mitochondria. Although in general, the distribution patterns showed overall similarities in the majority of instances, the proportions of total lipid within the supernatant of embryos and chicks from young parents were noticeably lower and the proportions of total lipid within the mitochondria noticeably higher compared with offspring from mature parents.

4.2 Heart.

During the embryonic and post-hatch periods lipid was preferentially associated with the mitochondrial and microsomal fractions. As in the liver, the proportions of lipid associated with the mitochondria increased following hatching. In comparison with embryos from mature parents, those from young parents displayed higher associations of lipid within the mitochondria and compensatory decreases in microsomal levels; this was also true of the chicks immediately following hatching.

4.3 Brain.

In contrast to the liver and heart, the proportions of lipid associated with the supernatant from the brain were very low, throughout the embryonic and post-hatch periods. The proportions of lipid associated with the mitochondria were markedly higher than those displayed by the liver and heart. Any differences in distribution between embryos and chicks from young parents were less noticeable than the liver and heart.

5 Major lipid moieties in the subcellular fractions of the liver, heart and brain.

The relative concentrations (expressed as mean percentages per unit weight of total lipid) of the major lipid components of the mitochondria in the liver, heart and brain, of embryos and chicks from mature and young parents, are given in Tables 15, 17 and 19 respectively, the microsomes in Tables 21, 23 and 25 and the supernatants, in Tables 27, 29 and 31. The statistical significances of the changes in concentrations of the lipid components with increasing embryonic and post-hatch age and the differences between the embryos and chicks from mature and young parents, are given in Tables

16a b, 18a b, 20a b (mitochondria); 22a b, 24a b, 26a b (microsomes); 28a b, 30a b, 32a b (supernatant).

5.1 Mitochondria.

Phospholipids comprised by far the largest component in the mitochondria of all three tissues throughout the embryonic and post-hatch periods and were also accompanied by substantial proportions of free cholesterol. During embryonic development, relative proportions of all other lipid components were low, especially in the brain mitochondria. In embryos from mature parents, apart from some minor changes, the lipid composition of the mitochondria remained constant over the last week of incubation. Triglyceride concentrations in the mitochondria of the liver and heart which were low during embryonic development, showed a considerable increase following hatching. At the same time, the mitochondria from both tissues showed a decrease in their concentrations of cholesterol ester, whilst in the liver mitochondria, a decrease in the concentrations of phospholipids and free cholesterol was also shown. In the brain mitochondria there were marked changes in the concentrations of phospholipid and free cholesterol between days 1 and 6 of the post-hatch period.

Compared with the liver mitochondria of embryos and chicks from mature parents, those from young parent stock displayed significantly higher proportions of phospholipid and lower proportions of free cholesterol during the embryonic period and following hatching. Relative concentrations of cholesterol ester decreased immediately after hatching and the subsequent accumulation of triglycerides was very much reduced. There were no major differences in the lipid compositions of the mitochondria of brain from embryos and post-hatch chicks of mature and young parents. Following hatching, the subsequent accumulation of triglycerides was very much reduced in the heart mitochondria of chicks from young parents.

5.2 Microsomes.

As in the case of the mitochondria, phospholipids and free cholesterol were by far the largest components of the microsomal lipids during embryonic development. The proportions of other lipid components were particularly low in the microsomes of the brain. Relative concentrations of phopholipid in the microsomes of the liver in particular, were lower than those displayed by the mitochondria and the concentrations of free cholesterol were higher. Following hatching, again as in the mitochondria, triglycerides became a major component in the liver and heart microsomes and were accompanied by a reduction in the relative concentrations of cholesteryl esters. At hatching, the microsomes of the liver displayed a sharp increase in the concentration of phospholipids, whereas in the microsomes of the brain, there were marked changes in the concentrations of phospholipid and free cholesterol between days 1 and 6 of post-hatch. Apart from minor differences in the concentrations of triglycerides and phospholipids within the liver and heart post-hatch, the microsomal lipids of the embryos and chicks from young parents showed no major differences from those of mature parents.

5.3 Supernatant.

A feature of the supernatant fractions of the liver and the heart during embryonic development was the high concentrations of triglyceride and cholesterol ester, compared with the membranous components. Although there were high proportions of these components within the supernatant of the brain, their concentrations were very much lower than those within the supernatants of the liver and heart. Relative concentrations of cholesterol ester increased considerably during embryonic development, so that in the supernatant of the liver, by day 19 of incubation, they accounted for more than 60 per cent of the total lipid present. The increased

concentrations of cholesterol ester in the liver supernatant were accounted for, in the main, by a decrease in the concentrations of triglyceride and free cholesterol. In the supernatants of the liver and heart following hatching, there was a dramatic fall in the proportions of cholesterol ester and an equally dramatic rise in the proportions of triglyceride, whilst in the supernatant of the brain following hatching, concentrations of free cholesterol increased and those of phospholipid decreased. The pattern of lipid changes displayed in the supernatants from the liver and brain of both embryo and chicks from young parents were similar to those from mature parents. The supernatant in the heart tissue from embryos of young parents, however, showed distinctly lower proportions of triglyceride at the time of hatch.

6 The distribution of total lipid moieties between the mitochondria, microsomes and cellular supernatant in the liver, heart and brain.

6.1 Liver.

The distribution of the major lipid components between the three subcellular fractions of the liver of embryos and chicks from mature and young parents, are shown in Figures 8 and 9 respectively. As can be seen, the phospholipids were specifically and consistently associated with the membranous components during both the embryonic and post-hatch periods. The most notable feature was a sharp increase following hatching, in the proportion of the phospholipids associated with the mitochondria. Free cholesterol was also predominantly associated with the membranous components, in particular with the microsomes during the early part of the incubation period, but latterly and during the post-hatch period, with both the mitochondria and microsomes. The proportion of free cholesterol associated with the supernatant showed a consistent decline throughout both the incubation and post-hatch periods. Although somewhat variable compared with the phospholipids and free cholesterol, there was a far more

Figure 8

The distribution of the major lipids between the three subcellular fractions of the livers of embryos and chicks from mature parents.

(i) phospholipids
(ii) free cholesterol
(iii) triglycerides
(iv) cholesteryl esters

 mitochondria
 microsomes
 supernatant

Figure 8 (i)

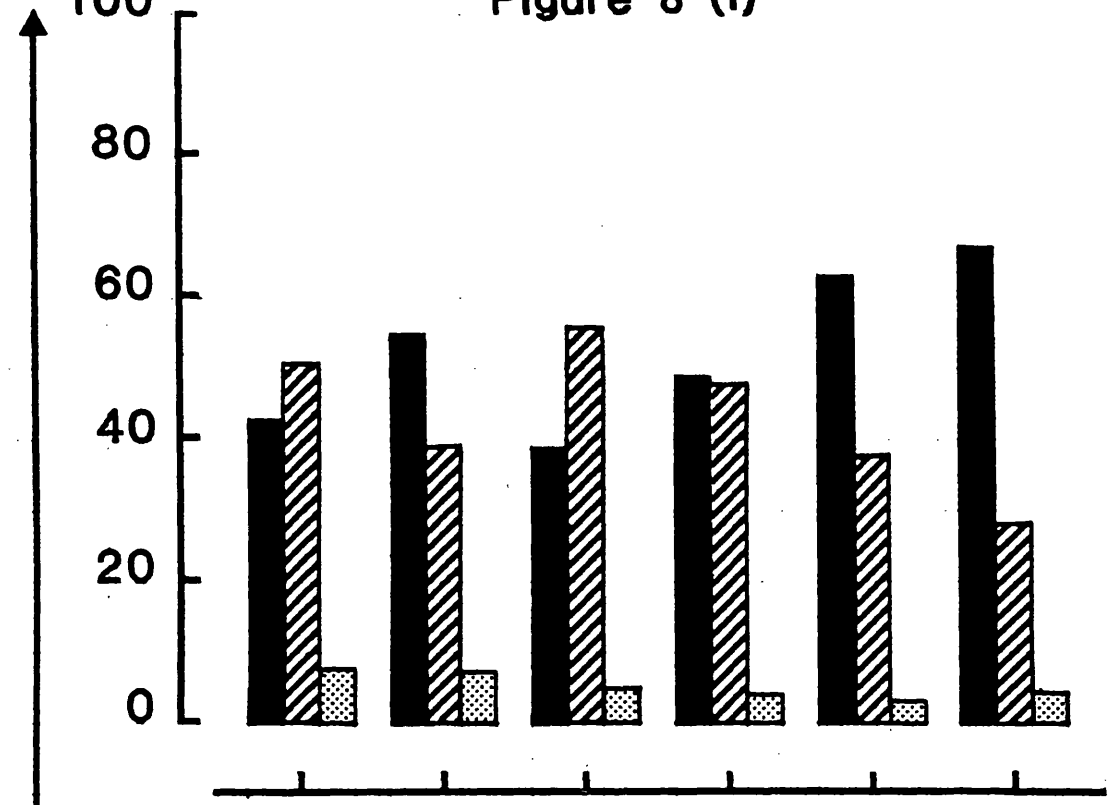


Figure 8 (ii)

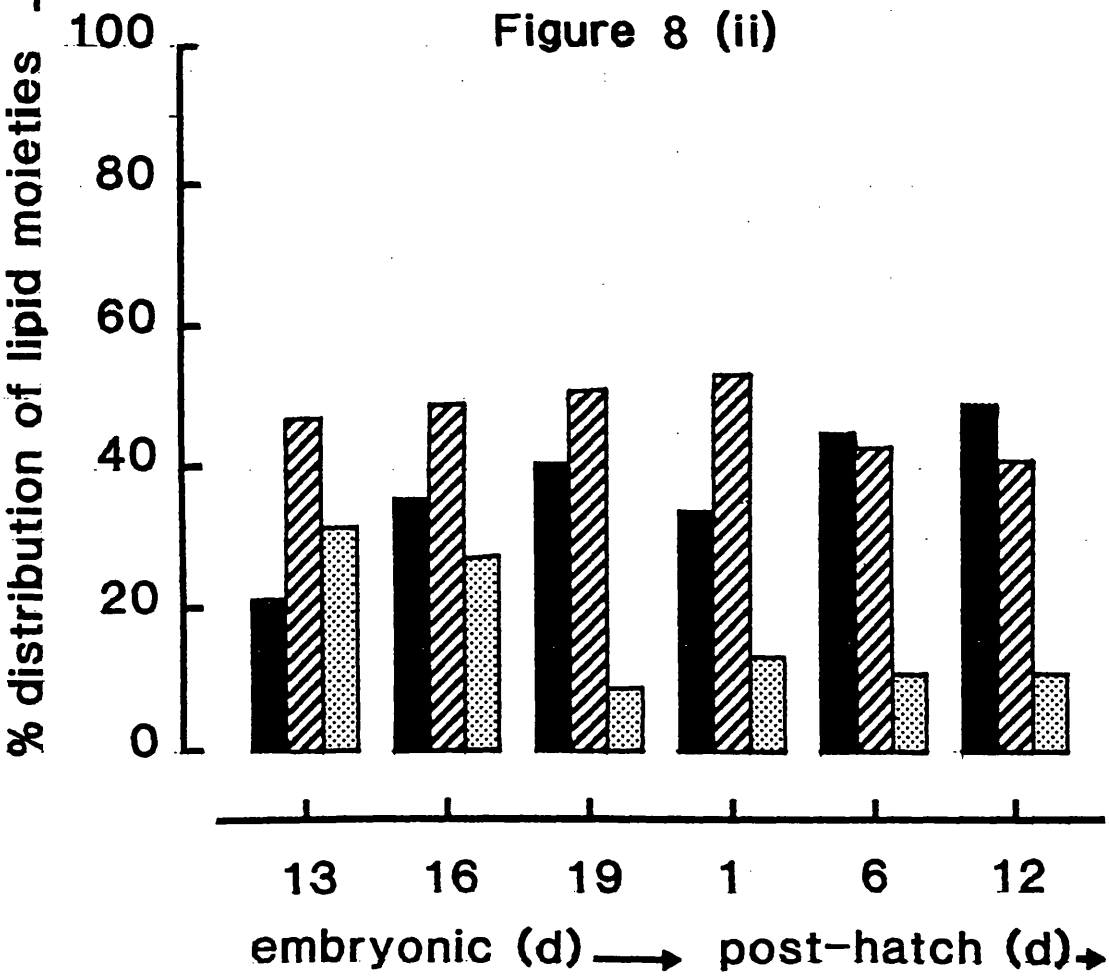


Figure 8 (iii)

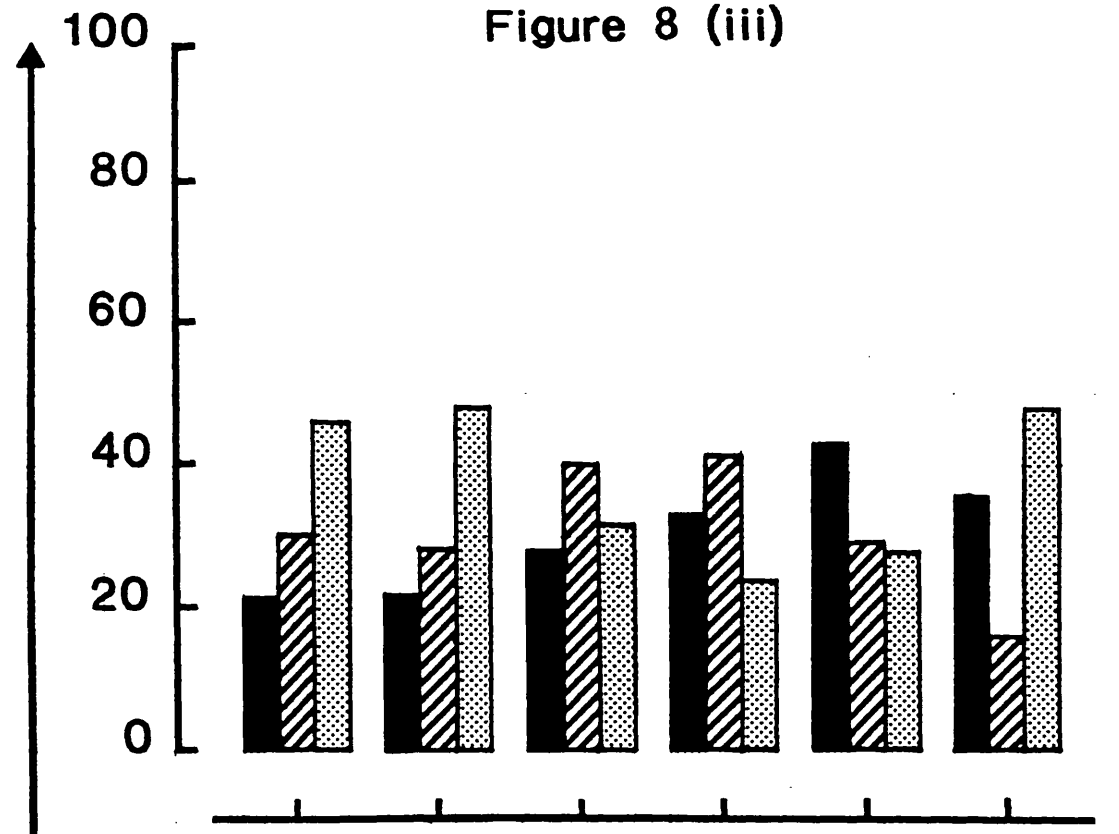


Figure 8 (iv)

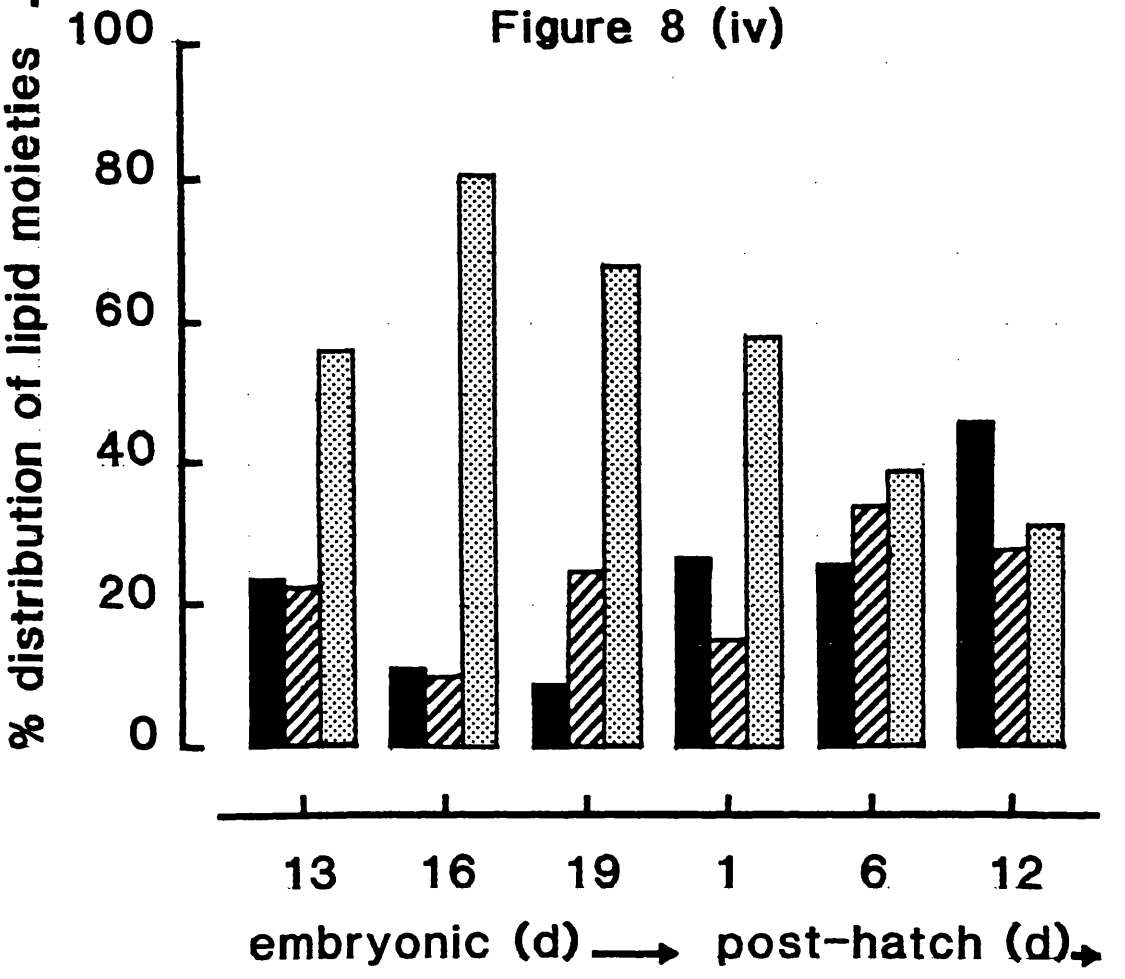


Figure 9

The distribution of the major lipids between the three subcellular fractions of the livers of embryos and chicks from young parents.

(i) phospholipids
(ii) free cholesterol
(iii) triglycerides
(iv) cholesteryl esters

 mitochondria
 microsomes
 supernatant

(NA) no data available

Figure 9 (i)

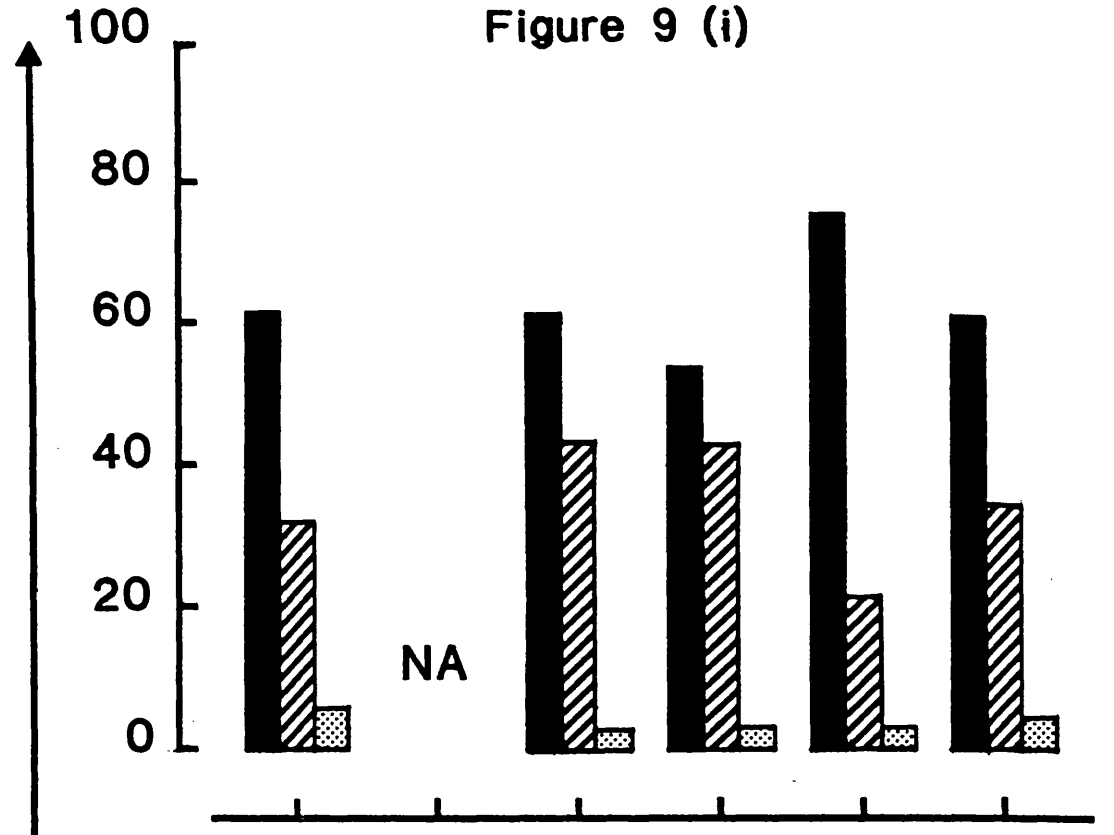


Figure 9 (ii)

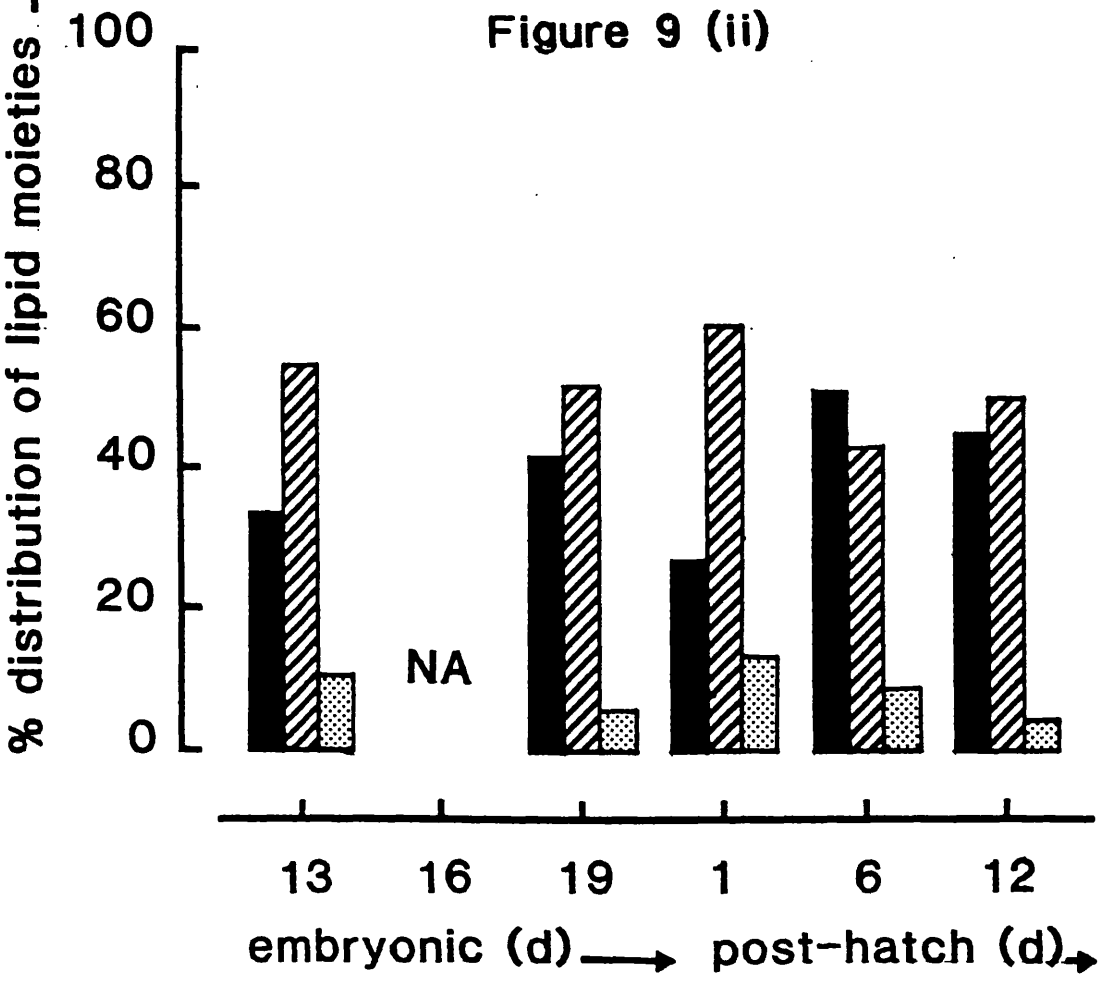


Figure 9 (iii)

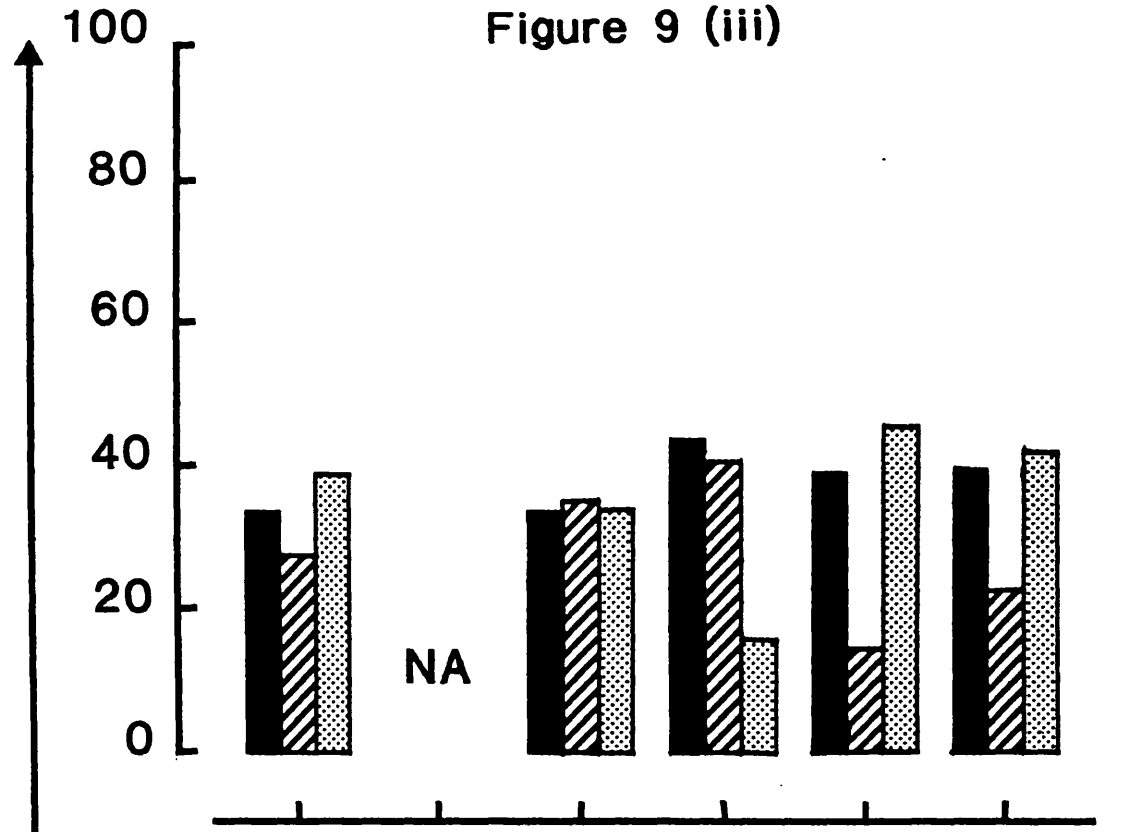
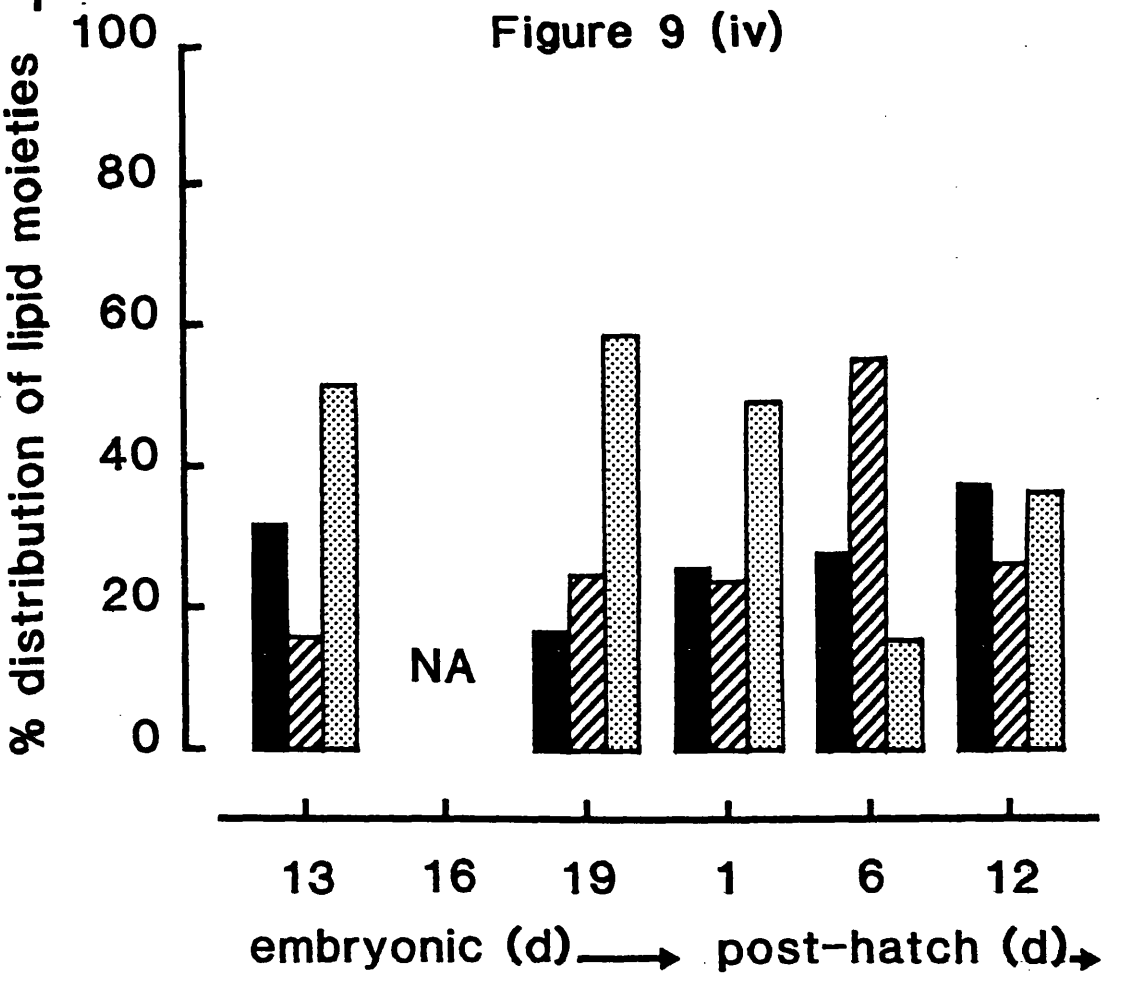


Figure 9 (iv)



even distribution of triglycerides between the three subcellular components over the embryonic and post-hatch periods. Cholesteryl esters showed a highly specific association with the supernatant fractions up to and including the first day of post-hatch. Thereafter, the proportion associated with the mitochondria and microsomes increased markedly.

Although the overall distribution patterns between the subcellular components for the embryos and chicks from young parents were similar to those of the mature group, some notable differences were apparent. Thus, in the case of the phospholipids, there was a very pronounced preference for association with the mitochondria; the proportions of phospholipids and free cholesterol associated with the supernatant fraction were extremely low. Although there was still a marked association of cholesteryl esters with the supernatant fraction, the disparity in its distribution between the three subcellular components was far less marked than for the embryos and day-old chicks from mature parents.

6.2 Heart.

Figures 10 and 11 respectively show the distribution of the major lipid components between the three subcellular fractions of the heart of embryos and post-hatch chicks from mature and young parents. As in the case of the liver, the phospholipids showed a marked preferential association for the mitochondria and microsomes, particularly the former during the post-hatch period. By contrast, free cholesterol showed a preferential association with the microsomes. No consistent pattern was discernible for the distribution of triglycerides between the three subcellular components over both the embryonic and post-hatch periods. As in the case of the liver, cholesteryl esters showed a specific association with the supernatant fraction, a feature which was continued over the whole of the post-hatch period.

Figure 10

The distribution of the major lipids between the three subcellular fractions of the hearts of embryos and chicks from mature parents.

(i) phospholipids
(ii) free cholesterol
(iii) triglycerides
(iv) cholesteryl esters


mitochondria


microsomes


supernatant

Figure 10 (i)

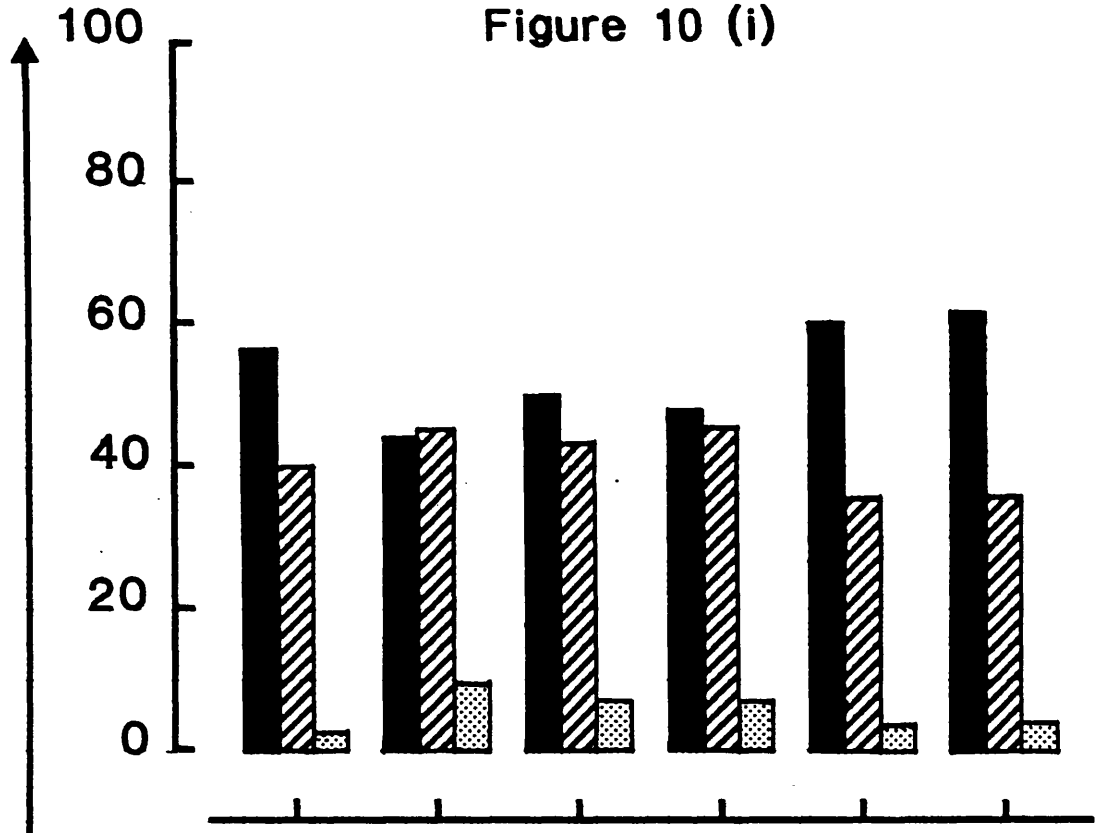


Figure 10 (ii)

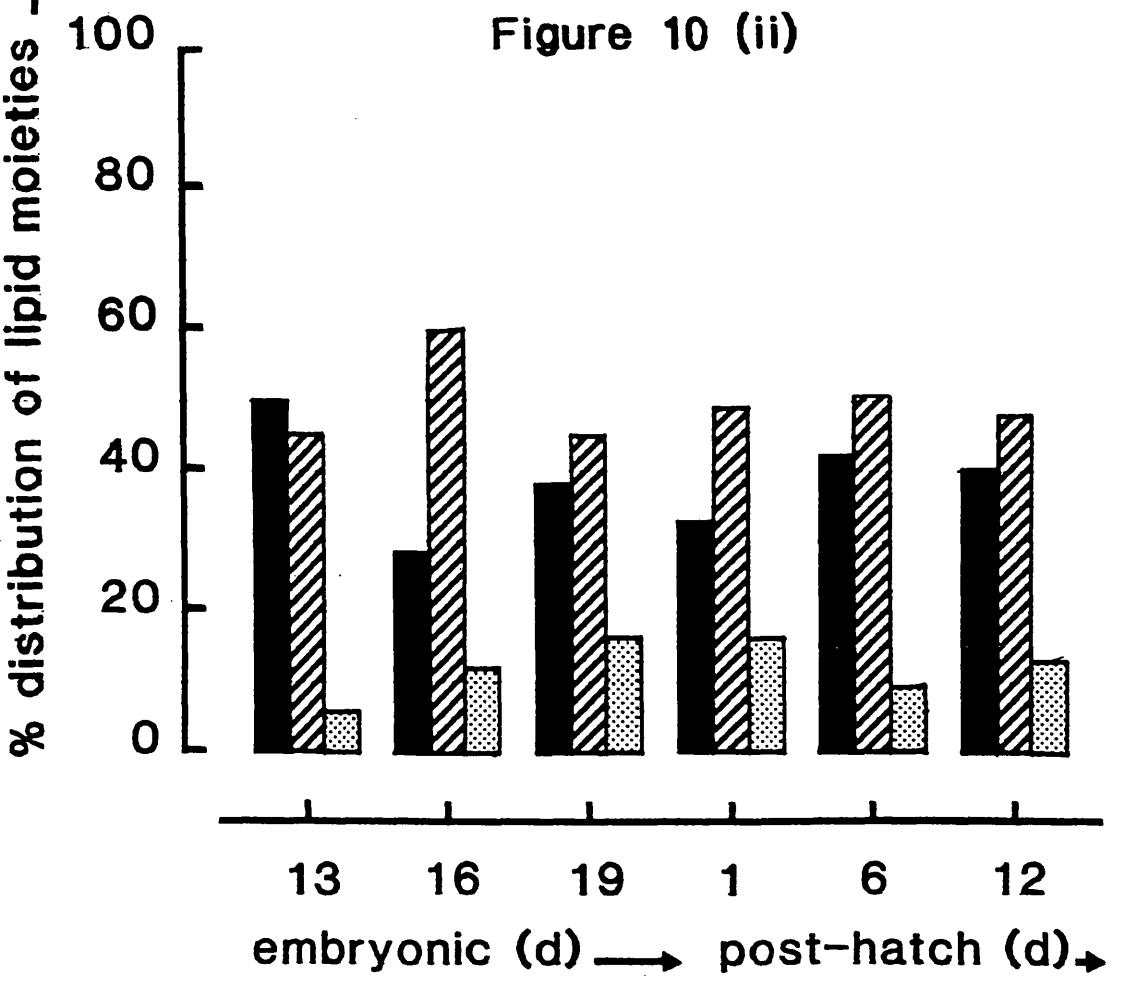


Figure 10 (iii)

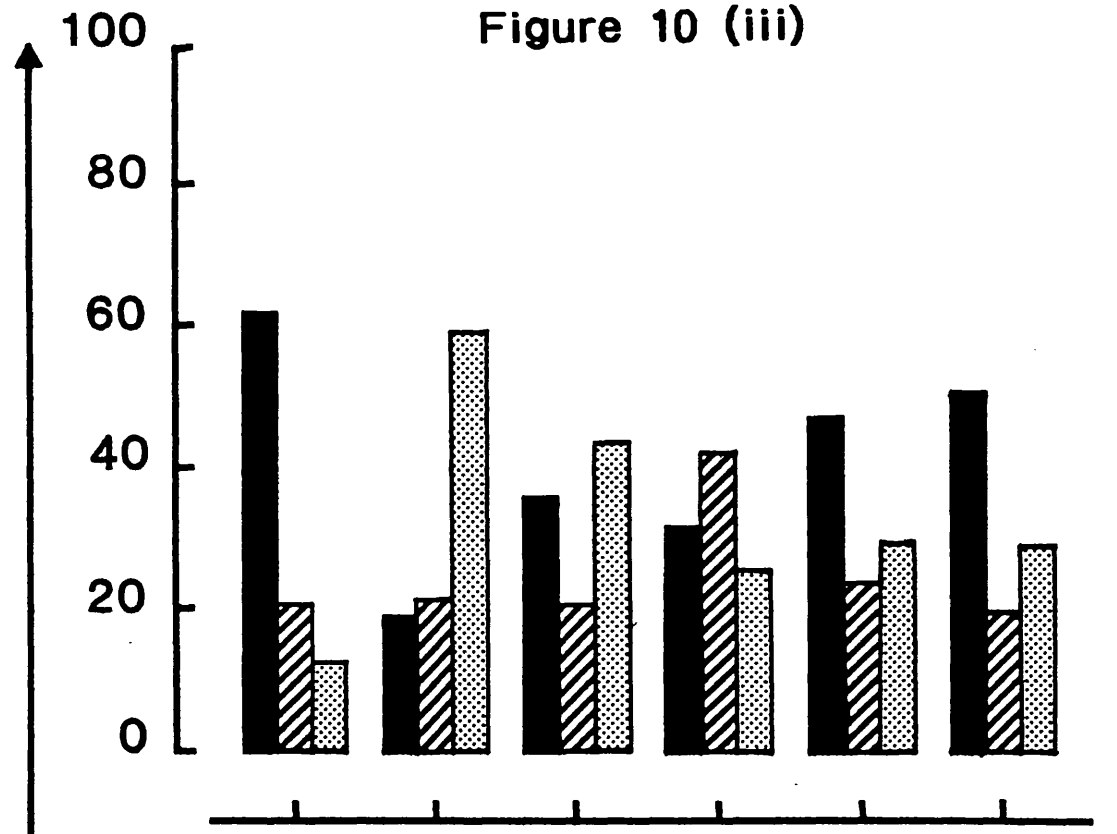


Figure 10 (iv)

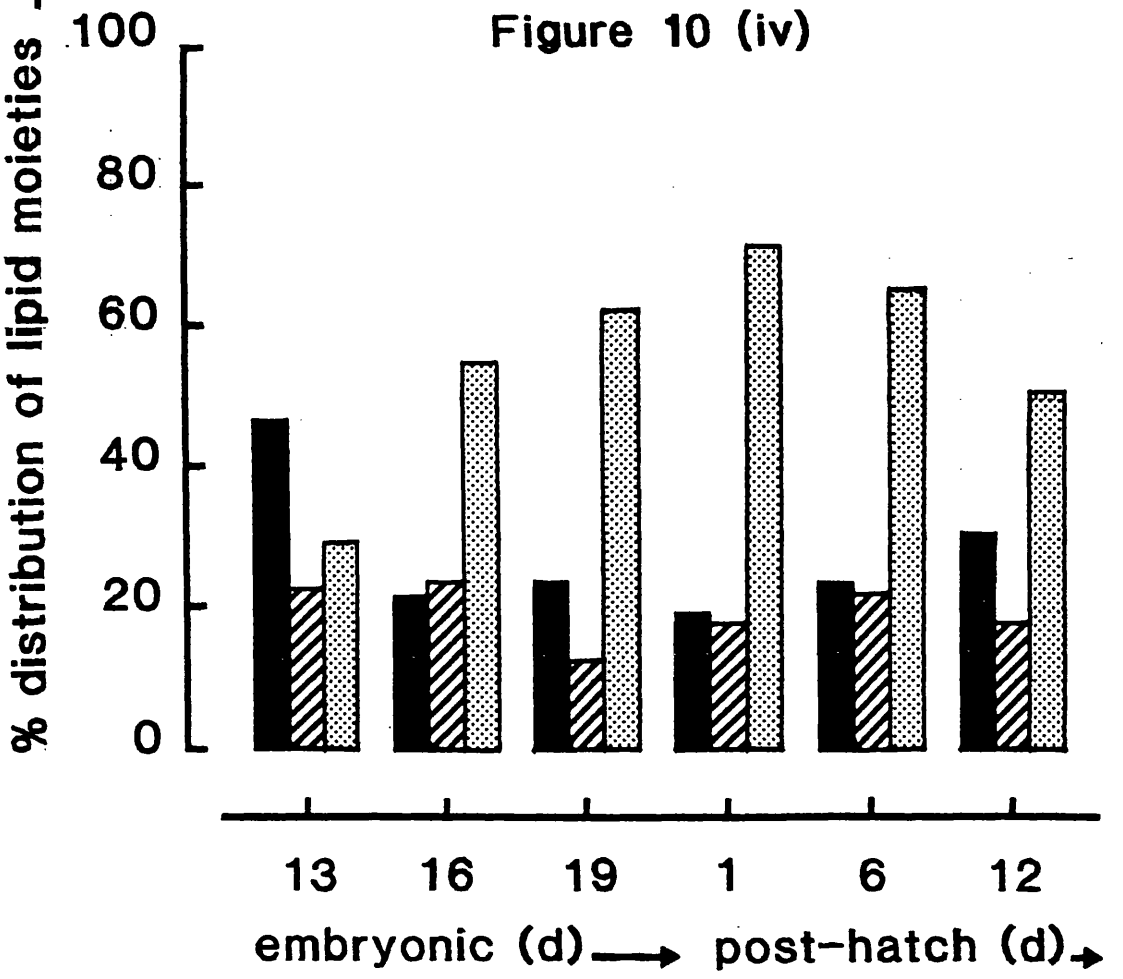


Figure 11

The distribution of the major lipids between the three subcellular fractions of the hearts of embryos and chicks from young parents.

(i) phospholipids
(ii) free cholesterol
(iii) triglycerides
(iv) cholesteryl esters

mitochondria
microsomes
supernatant

(NA) no data available

Figure 11 (i)

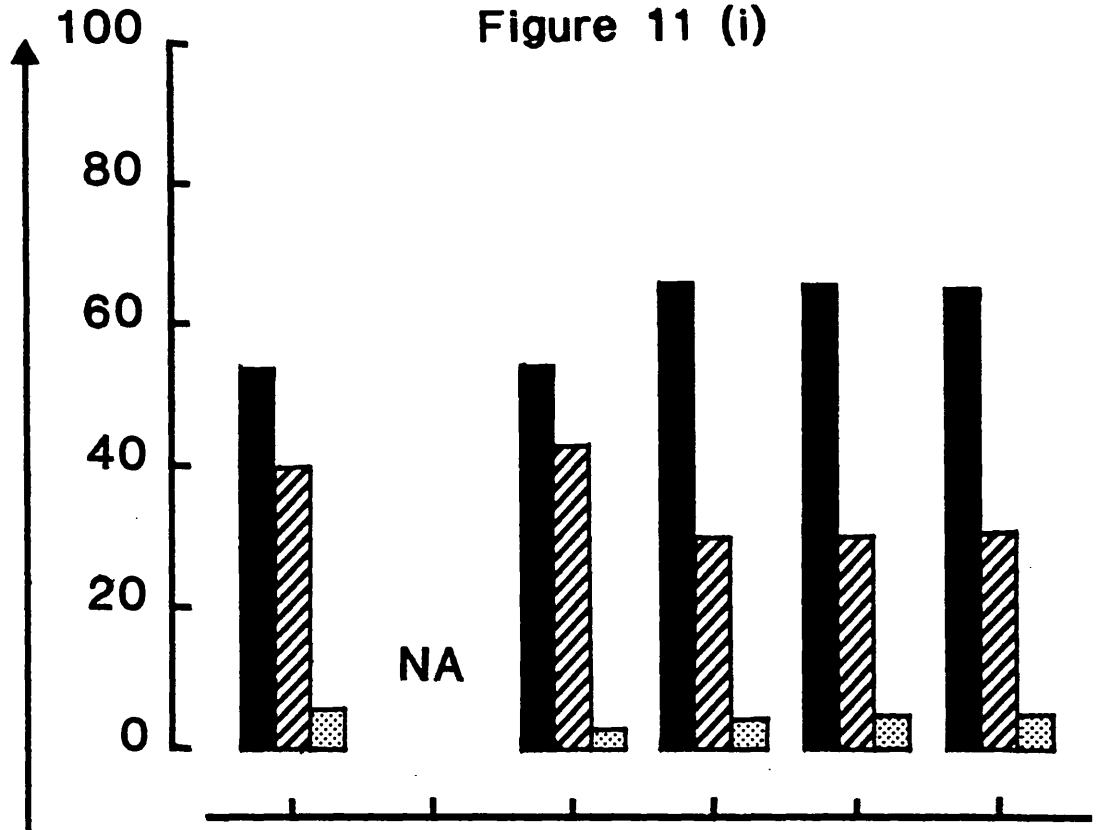


Figure 11 (ii)

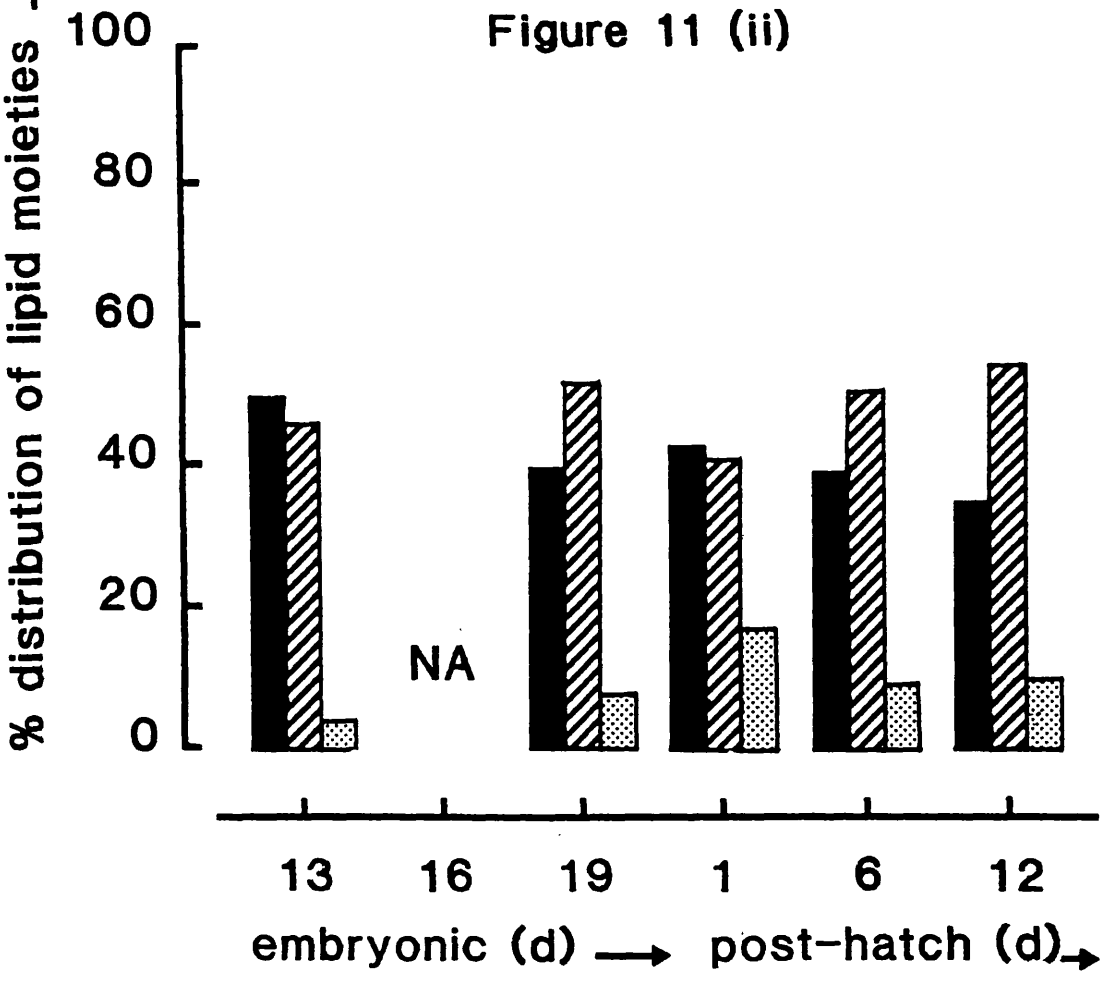


Figure 11 (iii)

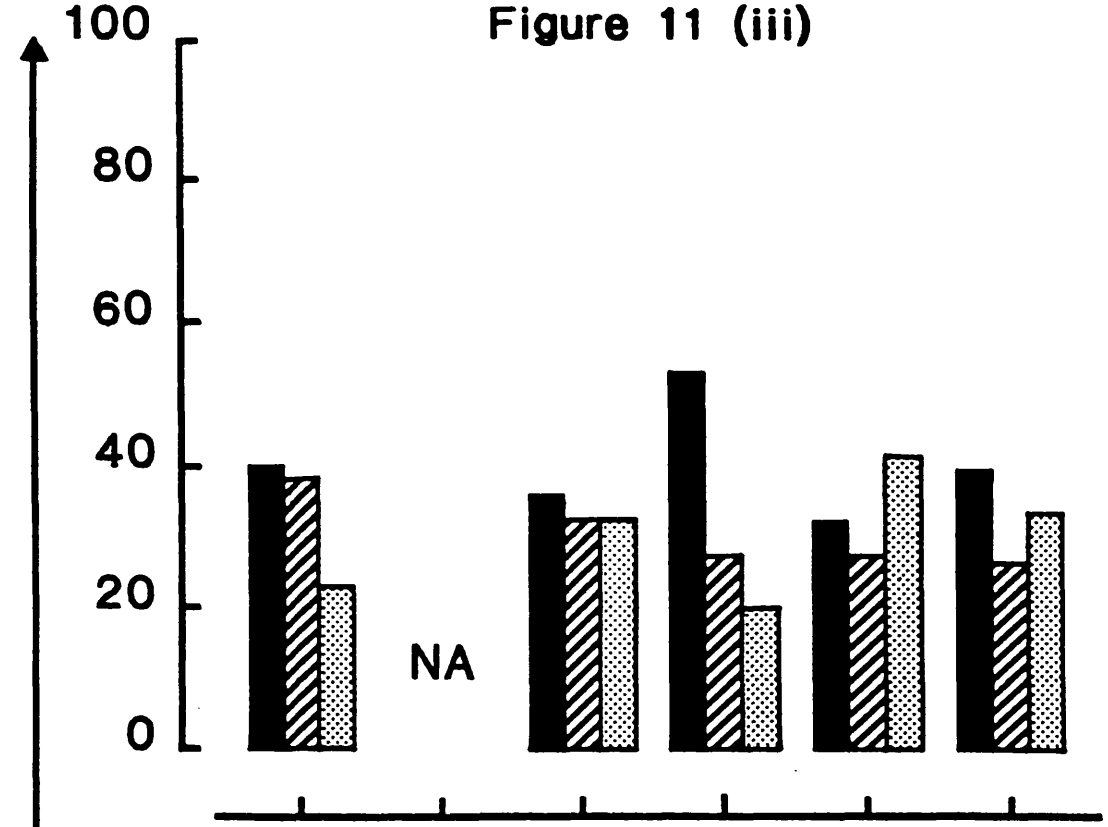
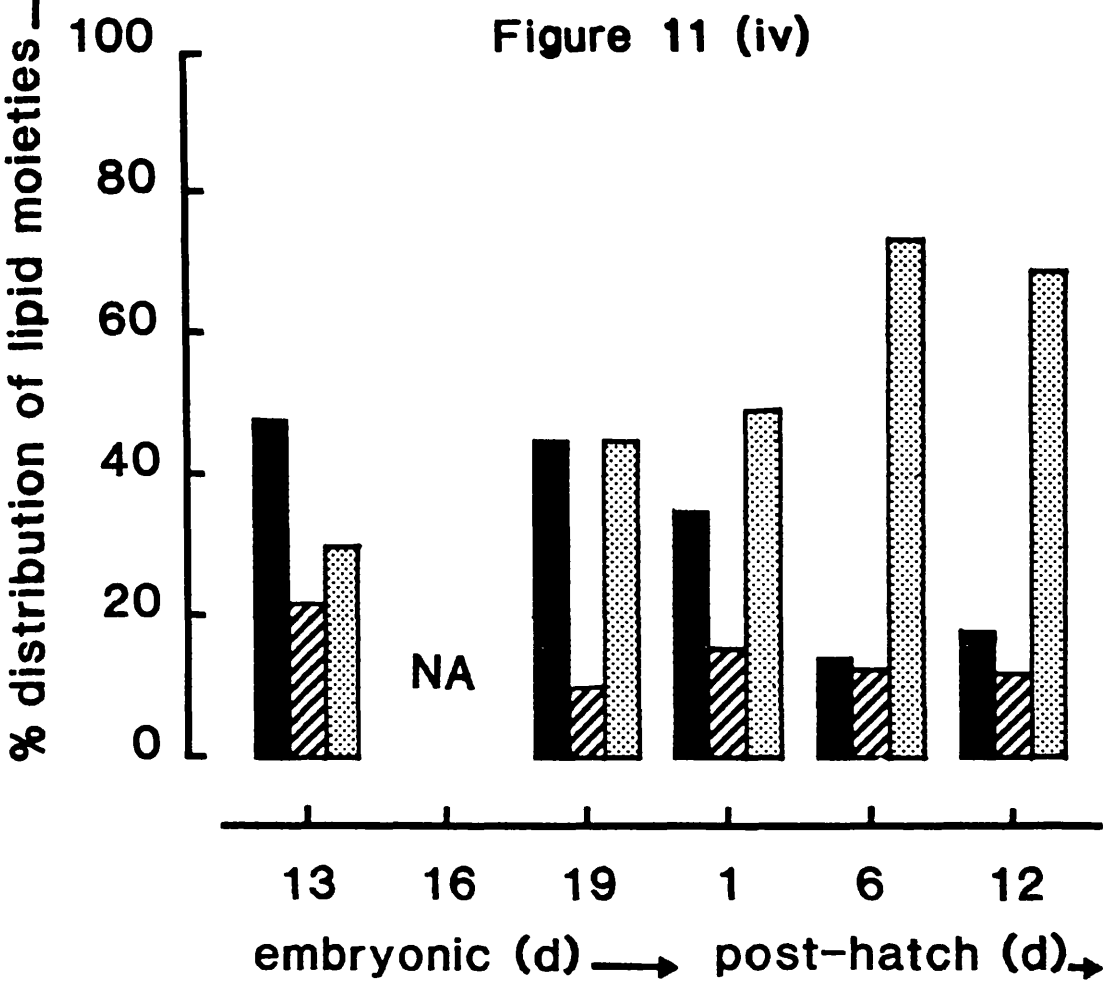


Figure 11 (iv)



In the embryos and chicks from young parents, the preferential association of phospholipids with the mitochondria was particularly marked; proportions of phospholipids associated with the supernatant were extremely low. The association of cholesteryl esters with the supernatant was much reduced during the embryonic period. By contrast, in chicks, immediately post-hatching, there was a predominant association of cholesteryl esters in the supernatant.

6.3 Brain.

The distribution of the major lipid components between the three subcellular fractions of the brain are shown in Figures 12 and 13. In contrast to the liver and heart, there was a marked similarity in the distribution of the lipid moieties between the cellular components with very little change occurring during the embryonic and post-hatch periods. Thus, in all cases, the lipid moieties were predominantly associated with the mitochondria in particular, and the microsomes. With increasing incubation and post-hatch time the association of the phospholipids and free cholesterol with the mitochondria increased with a concomitant decrease in their association with the microsomes. Whereas the proportions of phospholipids and free cholesterol associated with the supernatant remained very low throughout the embryonic and post-hatch periods, proportions of triglyceride and cholesterol ester associated with the supernatant were much higher. However, the proportions of total cellular cholesterol ester associated with the supernatant fractions of the brain were very much lower than those demonstrated by the liver and heart. Compared with embryos and chicks from mature parents, those from young parents showed a particularly marked association of phospholipids and free cholesterol with the mitochondria; proportions of the other major lipid fractions associated with the supernatant were very low indeed.

Figure 12

The distribution of the major lipids between the three subcellular fractions of the brains of embryos and chicks from mature parents.

(i) phospholipids
(ii) free cholesterol
(iii) triglycerides
(iv) cholesteryl esters

 mitochondria
 microsomes
 supernatant

Figure 12 (i)

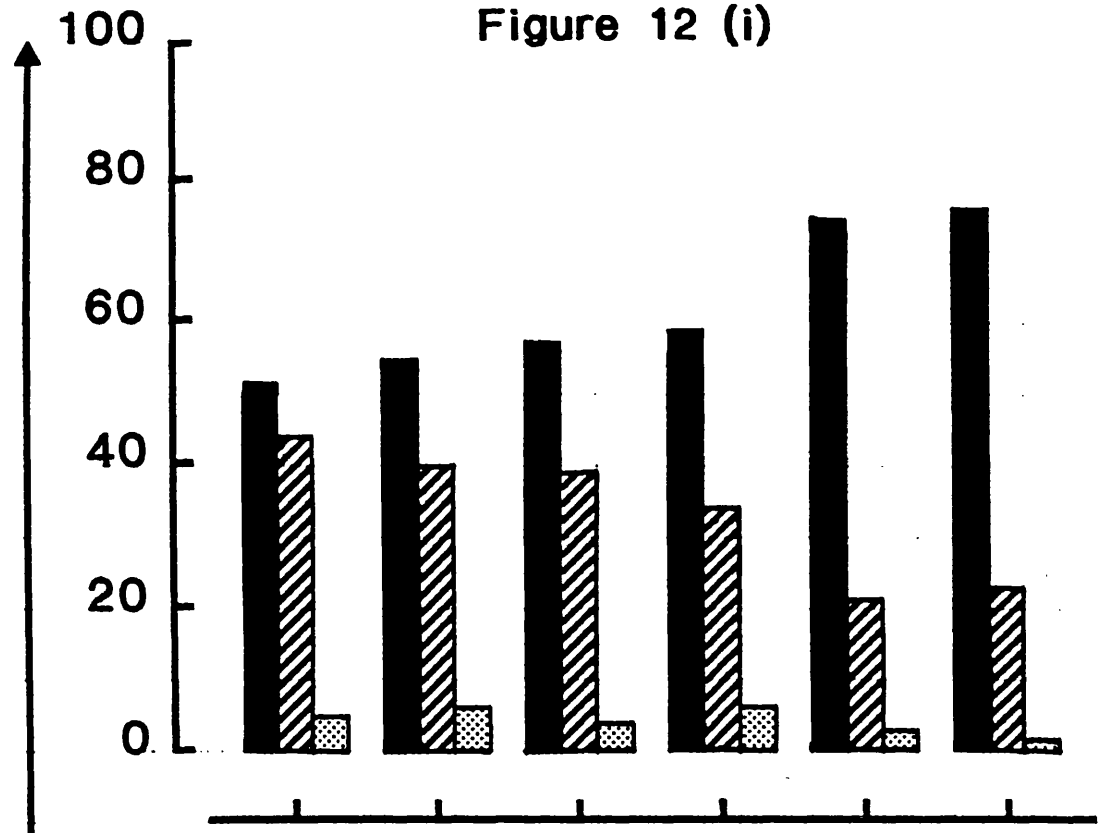


Figure 12 (ii)

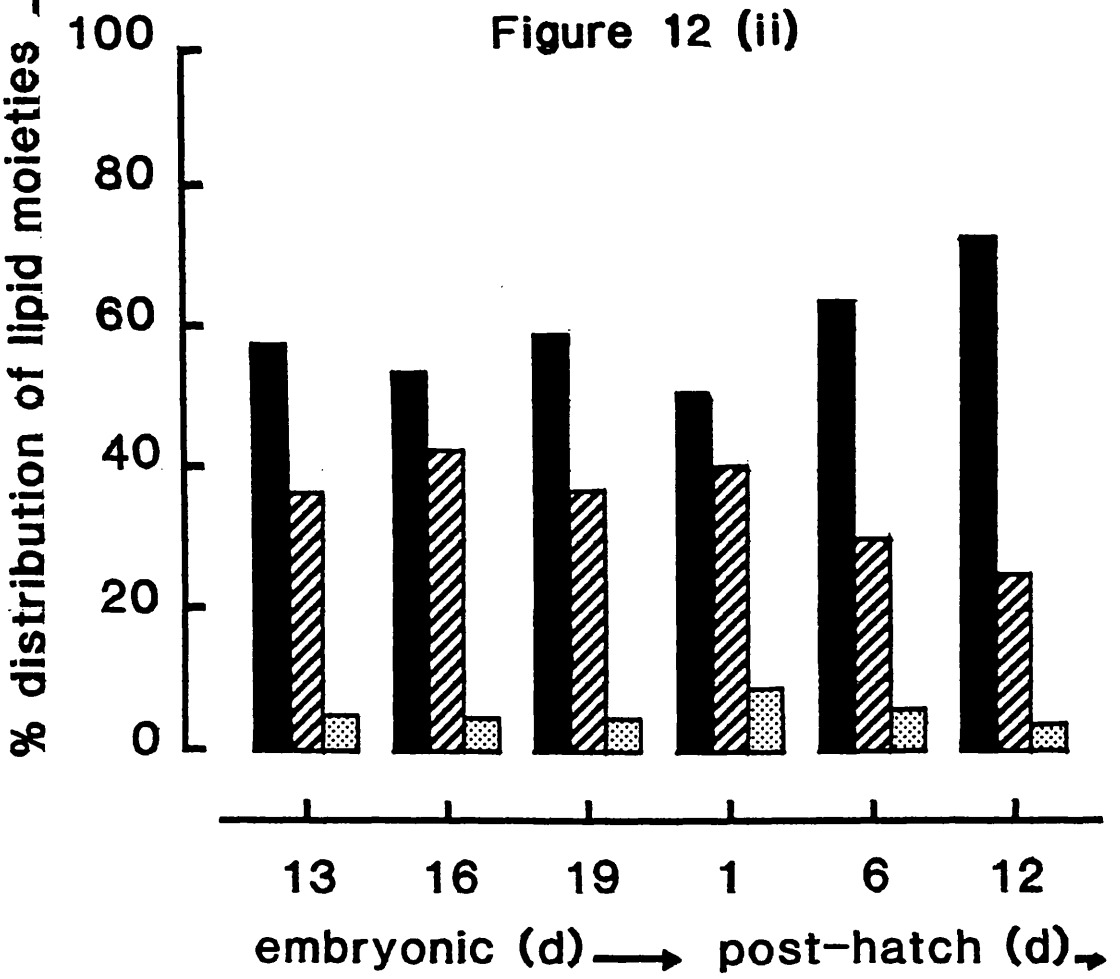


Figure 12 (iii)

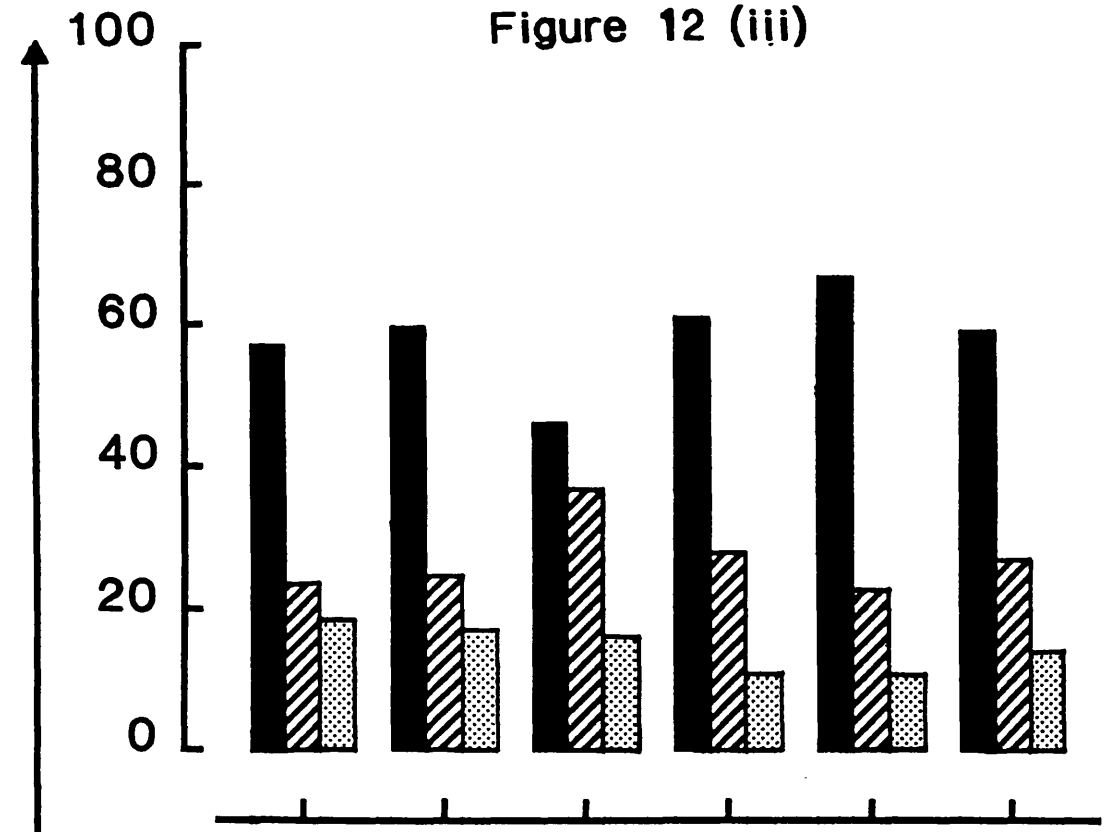


Figure 12 (iv)

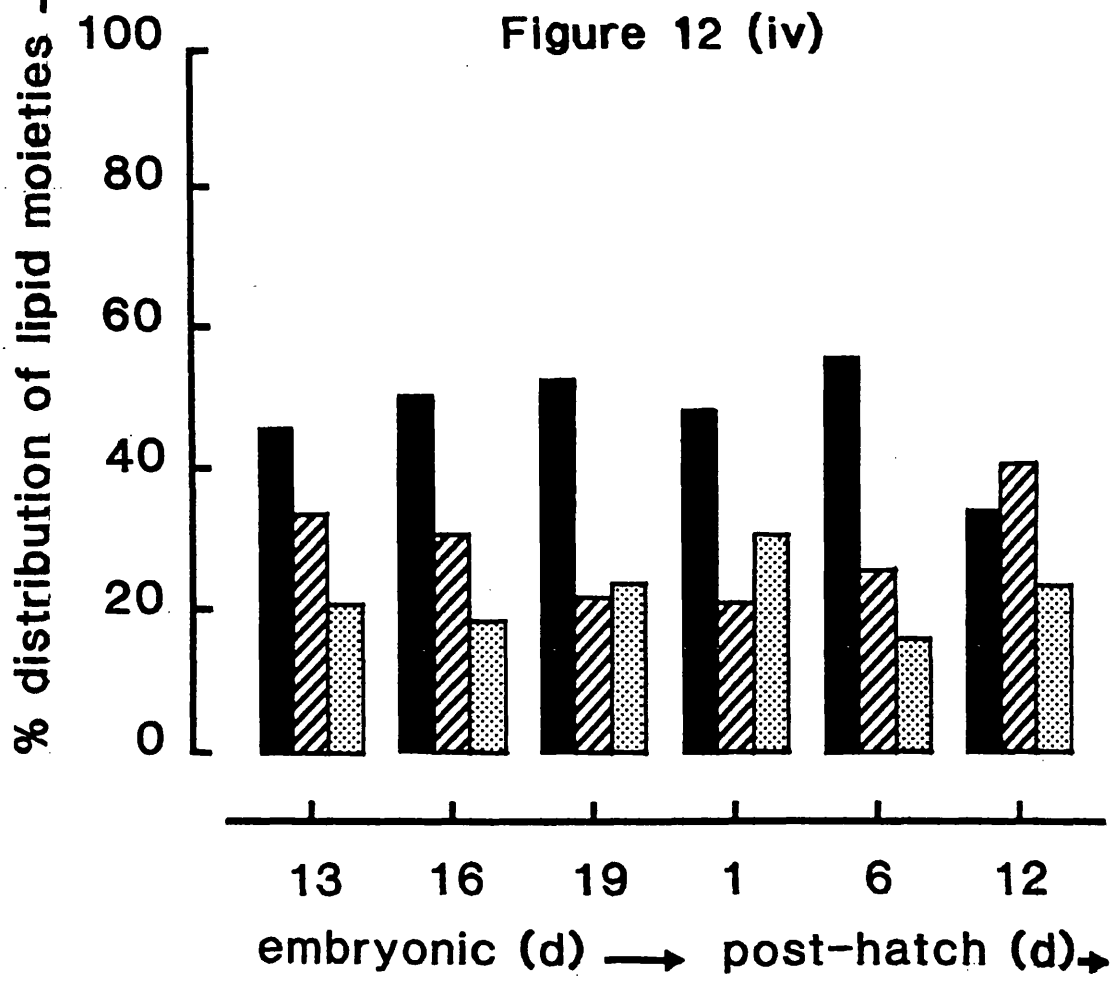


Figure 13

The distribution of the major lipids between the three subcellular fractions of the brains of embryos and chicks from young parents.

(i) phospholipids
(ii) free cholesterol
(iii) triglycerides
(iv) cholesteryl esters

 mitochondria
 microsomes
 supernatant

(NA) no data available

Figure 13 (i)

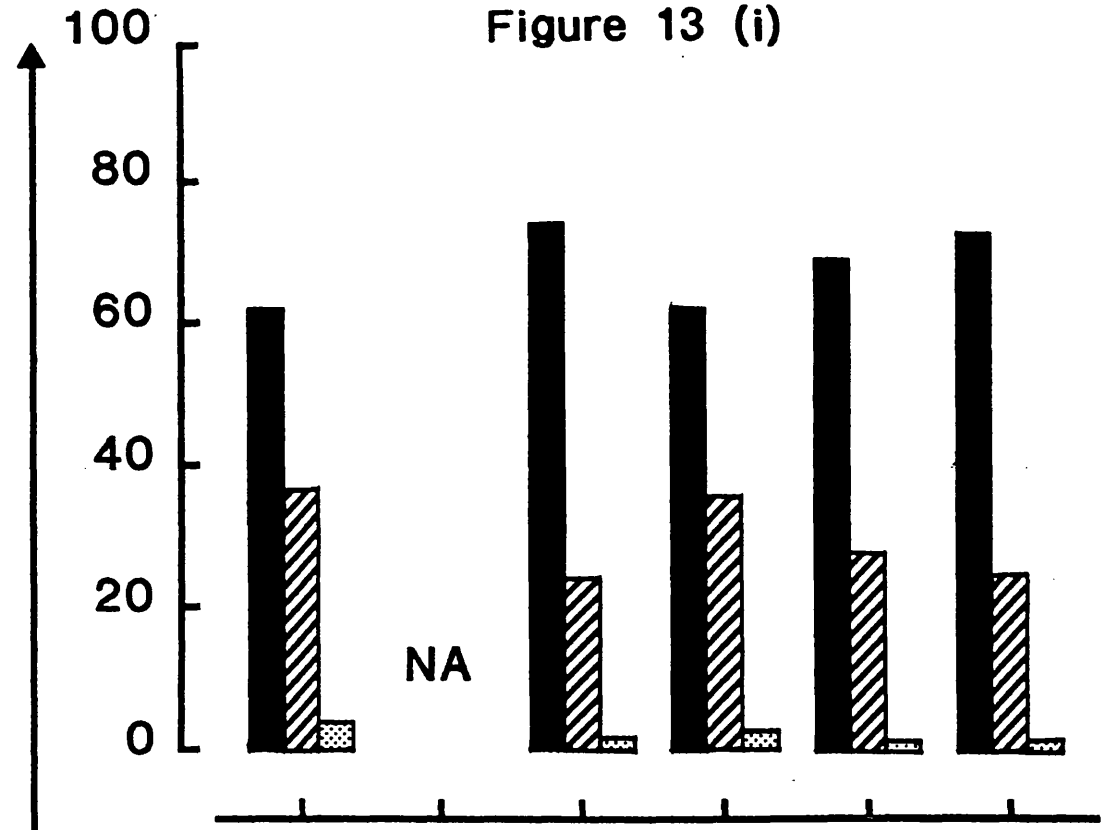


Figure 13 (ii)

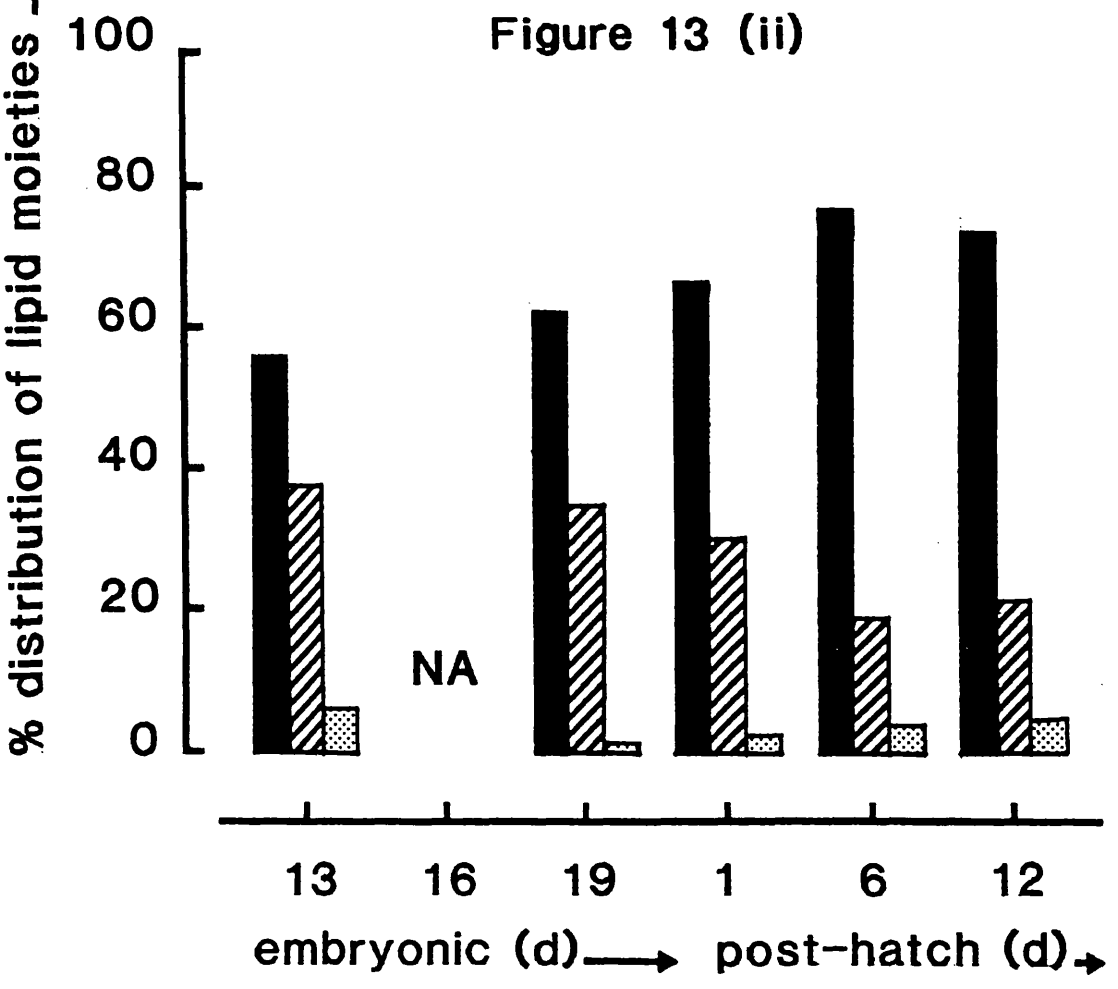


Figure 13 (iii)

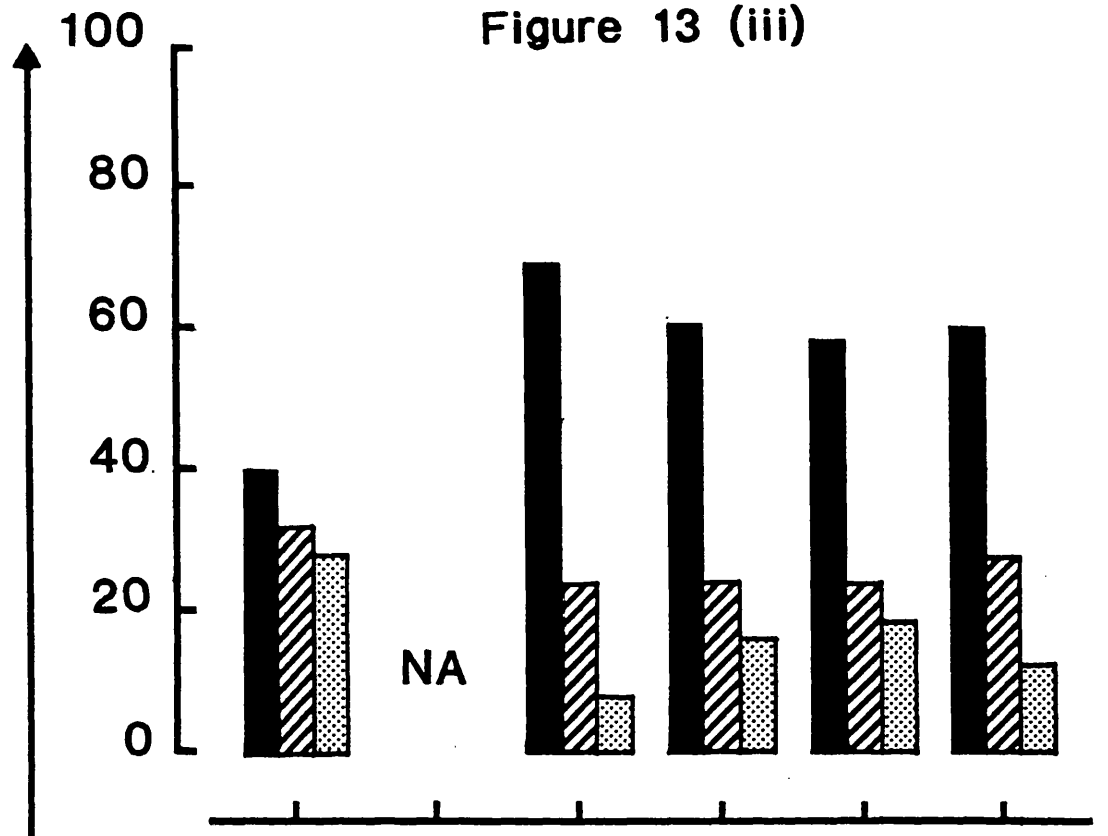
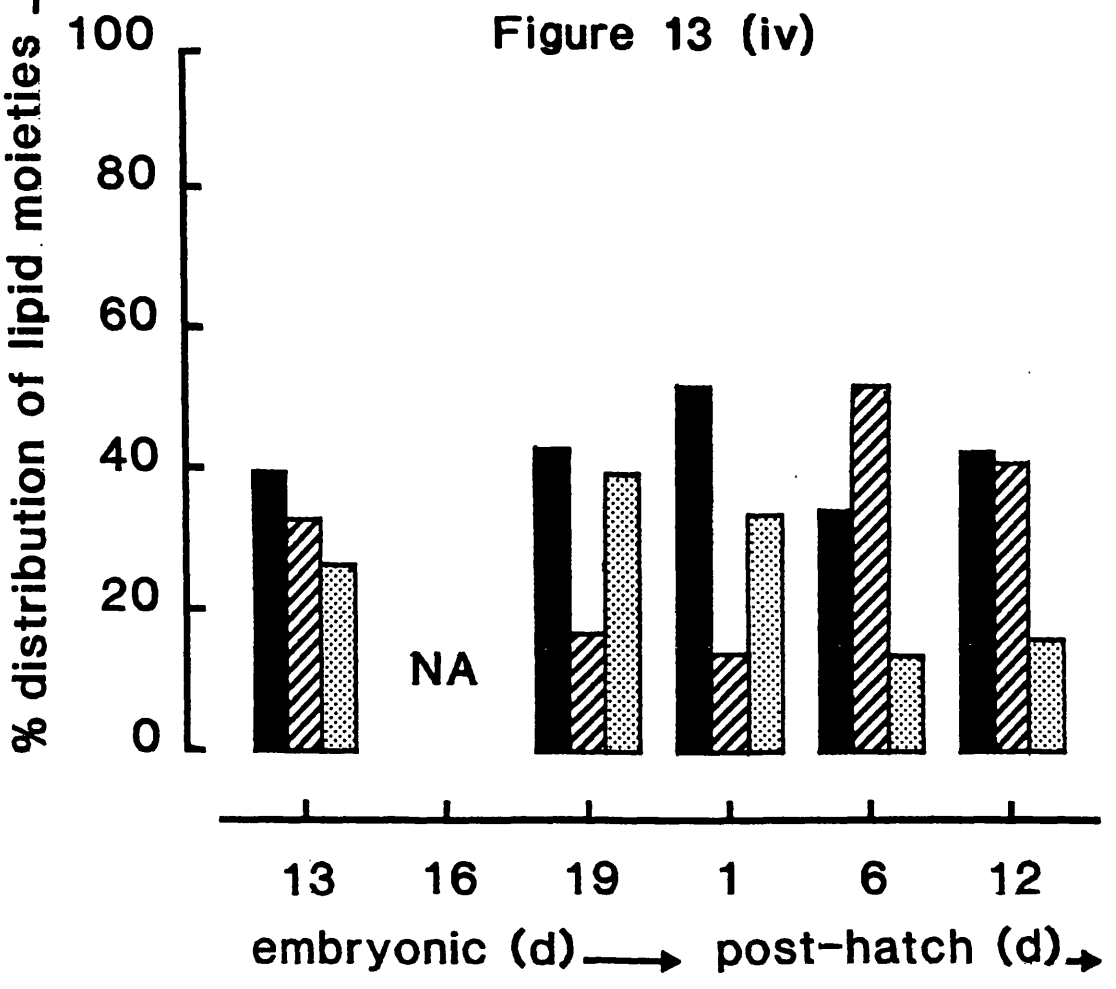


Figure 13 (iv)



Summary of sections 3-6

The major features of the lipid changes outlined in the above section may be summarised as follows:

1) Incubation was associated with an extensive lipid accumulation within the liver of the embryo during the last week of incubation and following hatching.

2) Tissue lipid accumulations were much lower in embryos and chicks from young parent stock.

3) Within the tissues, levels of lipid within the cells were highest in the mitochondria and microsomes.

4) In the liver, levels of lipid associated with the subcellular fractions all increased during the incubation period and were maintained post-hatching. In the heart and brain, lipid levels within the subcellular fractions remained virtually constant during both the incubation and post-hatch periods.

5) Embryos and chicks from young parent stock showed higher levels of lipid in the mitochondria of the tissues compared with progeny from mature parents.

6) Phospholipids accompanied by free cholesterol were by far the major lipid components of the mitochondria of the tissues. Whereas the mitochondria showed a stable lipid composition during incubation, following hatching, there were extensive changes. Relative concentrations of triglycerides increased within the mitochondria of the liver and heart and phospholipids within the mitochondria of the brain.

7) The mitochondria from the livers of embryos and chicks from young parent stock showed higher concentrations of phospholipids and lower concentrations of free cholesterol. Accumulations of triglyceride following hatching were very much lower.

8) Phospholipids and free cholesterol were the major lipid components of the microsomes but differed in proportion from the mitochondria. No major lipid compositional changes occurred during incubation. Following hatching, the microsomes of the liver and heart displayed accumulations of triglyceride. Changes in the concentrations of phospholipid and free cholesterol occurred within the brain. Accumulations of triglycerides following hatching were very much lower in the liver and heart microsomes from chicks of young parents.

9) The supernatant fraction displayed very high concentrations of cholesterol ester and triglyceride. Following hatching, the concentrations of cholesterol ester rapidly declined. Parental age was without effect on lipid composition.

10) In terms of total cell lipid, in the liver and heart, phospholipids and free cholesterol showed a predominant association with the membranous components and cholesteryl esters with the supernatant. Following hatching, proportions of cholesterol ester associated with the supernatant reduced markedly and proportions of phospholipid associated with the mitochondria increased. Lower proportions of cholesterol ester in the supernatant and higher proportions of phospholipid in the mitochondria were displayed by embryos and chicks from young parents. In the brain all the major lipids were predominantly concentrated in the membrane components with proportions associated with the mitochondria increasing during incubation and post-hatch.

7 Fatty acid compositions of the mitochondrial, microsomal and cellular supernatant fractions of liver, heart and brain tissue of the chick during embryonic and post-hatch development.

7.1 Phospholipids.

The relative concentrations (expressed as mean percentages per unit weight of total fatty acids) of the major fatty acids in the phospholipids of the mitochondria in the liver, heart and brain from embryos and chicks are given in Tables 33 34, 36 37, 39 and 40 respectively, the microsomes in Tables 42 43, 45 46, 48 and 49 and the supernatants, in Tables 51 52, 54 55, 57 and 58. The statistical significances of the changes in concentrations of the fatty acid components in the phospholipid fraction, with increasing embryonic and post-hatch age and the differences between the embryos/chicks from mature and young parents, are given in Tables 35a b, 38a b, 41a b (mitochondria); 44a b, 47a b and 50a b (microsomes); 53a b, 56a b and 59a b (supernatant).

7.1.1 Mitochondria.

As can be seen, palmitic and stearic acids accounted for between 40 and 50 per cent of the total fatty acids in the mitochondrial phospholipids of all three tissues throughout development. The concentrations of polyunsaturated fatty acids varied between the tissue mitochondria, although in general, those of the liver and heart were very similar to each other. In the phospholipid fraction of the liver mitochondria, by far the major unsaturated fatty acid present during the final week of incubation was arachidonic acid; the concentrations of oleic, linoleic and docosahexaenoic acids were all very

much lower during the embryonic period. The remaining identifiable unsaturated fatty acids represented less than 3 per cent of the total present. An obvious relationship existed between the variations in the concentrations of certain of the fatty acids in the phospholipid fraction of the liver mitochondria over the last week of incubation. Although the total saturated fatty acids made up a relatively constant proportion of the total fatty acids at each stage of development, there was a pronounced increase in the concentration of stearic acid and decrease in palmitic acid as the age of the embryo increased (Table 33; Figure 14); these changes were most pronounced in the final few days of incubation. A slight increase in the proportions of total unsaturated fatty acids within the mitochondria lipids occurred at this time. Within the polyunsaturated fatty acids, the proportions of linoleic acid increased and that of arachidonic acid decreased (Table 33; Figure 15). During the final week of incubation there were curvilinear relationships between the concentrations of palmitic and linoleic acids (Figure 16), palmitic and arachidonic acids (Figure 17), stearic and linoleic acids (Figure 18) and stearic and arachidonic acids (Figure 19). In the case of linoleic and arachidonic acids, these changes in proportions were most pronounced between days 13 and 16 of incubation. There was a pronounced increase in the concentration of docosahexaenoic acid between the nineteenth day of incubation and day 1 of post-hatch.

Following hatching, palmitic and stearic acids still accounted for more than 45 per cent of the total fatty acids but arachidonic acid was no longer the major unsaturated fatty acid. Thus, by day 6 of post-hatch, the concentration of arachidonic acid had decreased to a level of about one third of the value at hatching and had a concentration similar to that of docosahexaenoic acid. Following hatching therefore, the major unsaturated fatty acids by far were oleic and linoleic and as was the case during the embryonic period, the variations in relative concentrations of certain fatty acids were related. In contrast to that seen during embryonic development, there was a marked

Figure 14

The relationship between the concentrations (weight percentages of the total fatty acids) of palmitic (16:0) and stearic acids (18:0) in the phospholipids of the mitochondria (●) and microsomes (○) of chick embryo livers at days 13, 16 and 19 of incubation. The results are for embryos from mature parents.

Figure 15

The relationship between the concentrations (weight percentages of the total fatty acids) of linoleic (18:2) and arachidonic acids (20:4) in the phospholipids of the mitochondria (●) and microsomes (○) of chick embryo livers at days 13, 16 and 19 of incubation. The results are for embryos from mature parents.

Figure 14

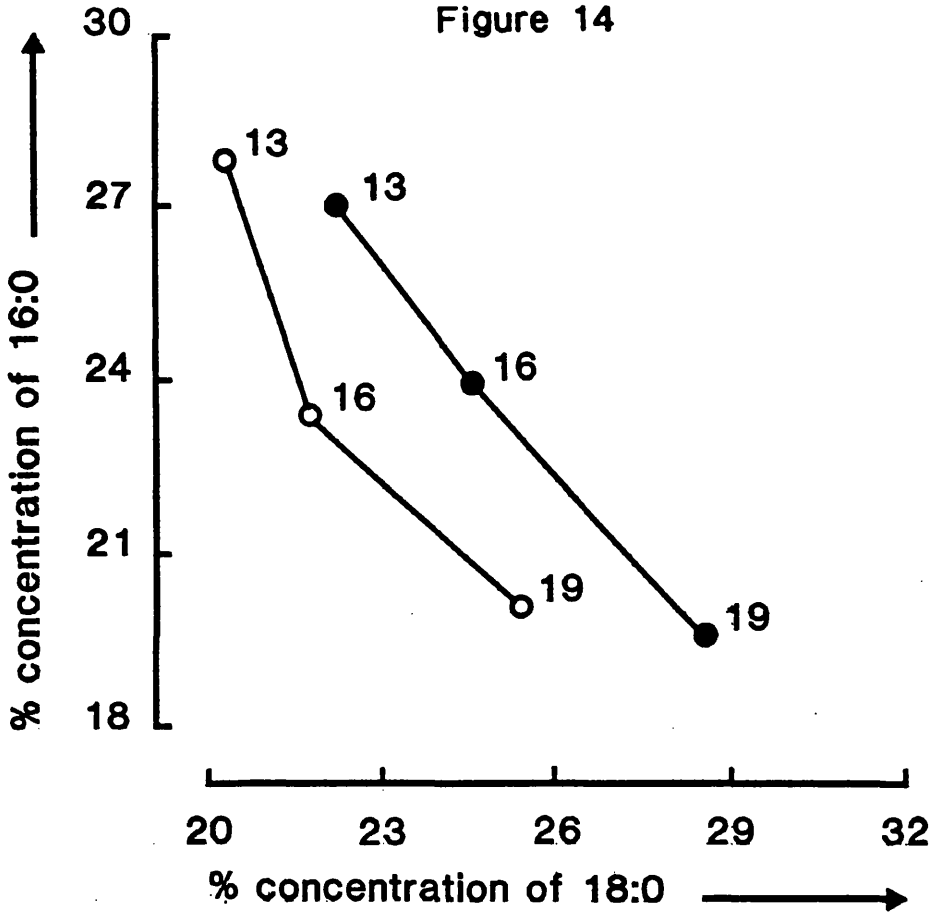


Figure 15

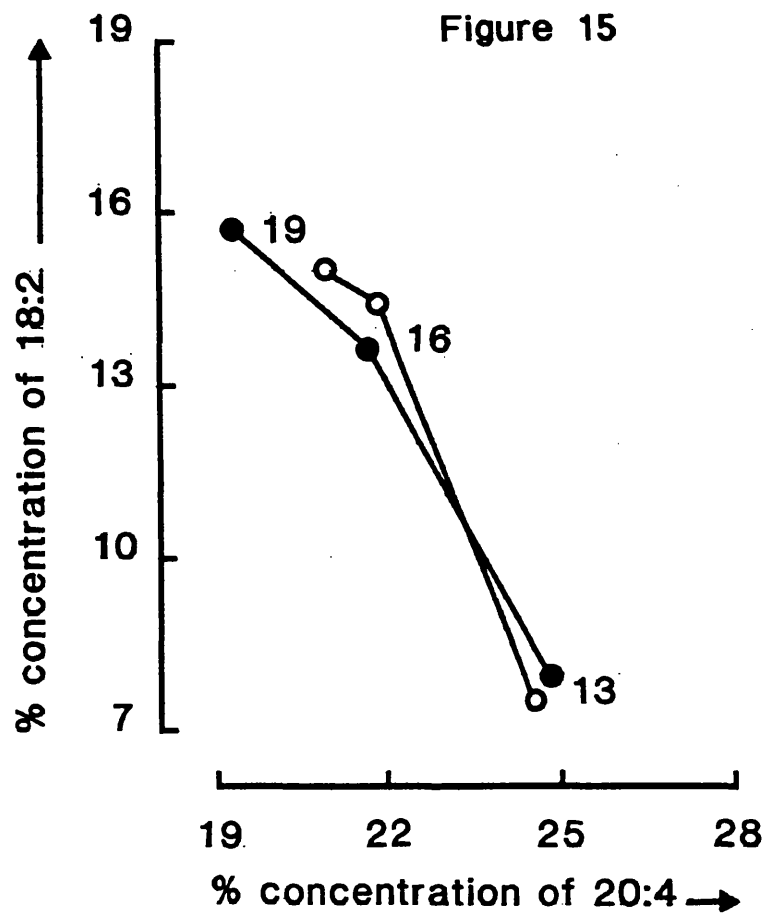


Figure 16

The relationship between the concentrations (weight percentages of the total fatty acids) of palmitic (16:0) and linoleic acids (18:2) in the phospholipids of the mitochondria (●) and microsomes (○) of chick embryo livers at days 13, 16 and 19 of incubation. The results are for embryos from mature parents.

Figure 17

The relationship between the concentrations (weight percentages of the total fatty acids) of palmitic (16:0) and arachidonic acids (20:4) in the phospholipids of the mitochondria (●) and microsomes (○) of chick embryo livers at days 13, 16 and 19 of incubation. The results are for embryos from mature parents.

Figure 16

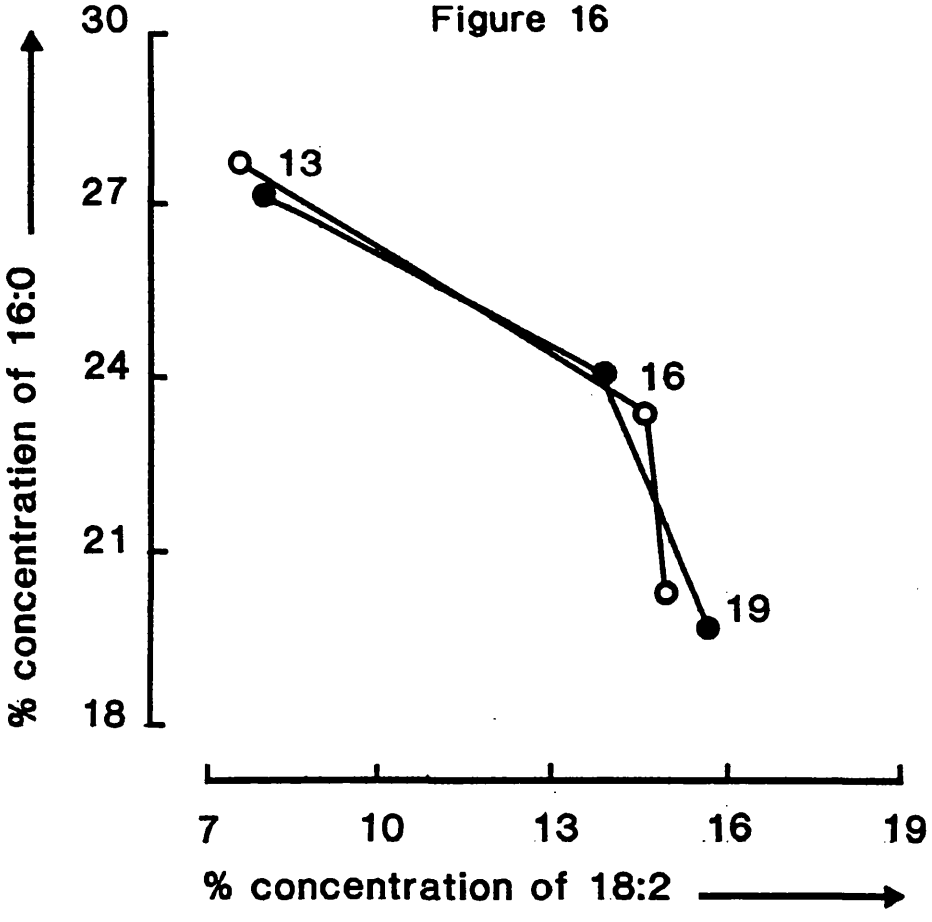


Figure 17

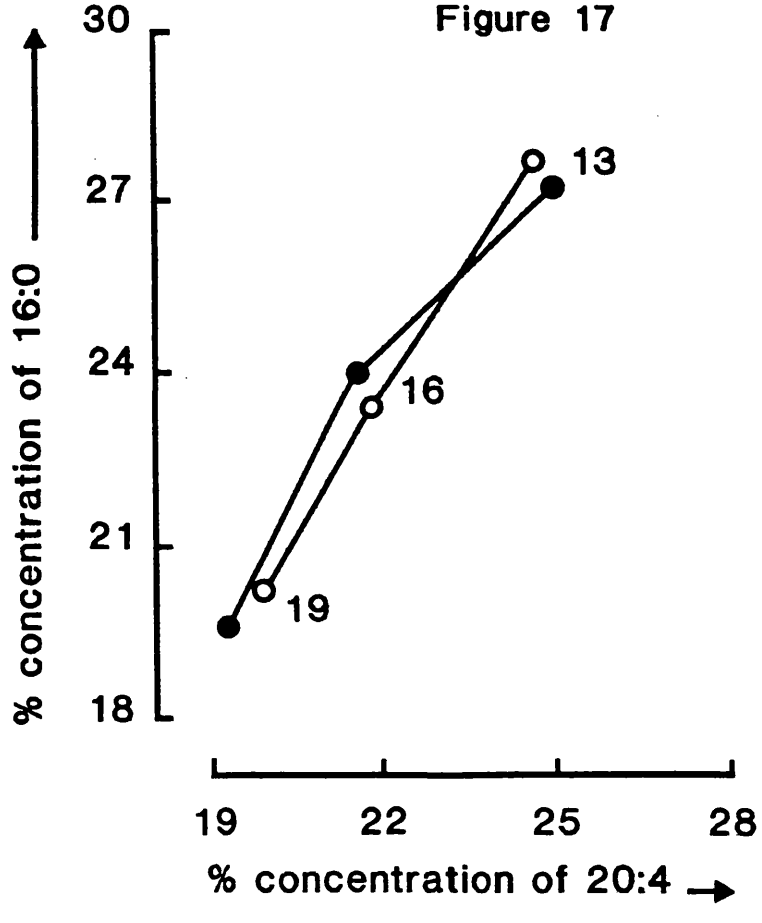


Figure 18

The relationship between the concentrations (weight percentages of the total fatty acids) of stearic (18:0) and linoleic acids (18:2) in the phospholipids of the mitochondria (●) and microsomes (○) of chick embryo livers at days 13, 16 and 19 of incubation. The results are for embryos from mature parents.

Figure 19

The relationship between the concentrations (weight percentages of the total fatty acids) of stearic (18:0) and arachidonic acids (20:4) in the phospholipids of the mitochondria (●) and microsomes (○) of chick embryo livers at days 13, 16 and 19 of incubation. The results are for embryos from mature parents.

Figure 18

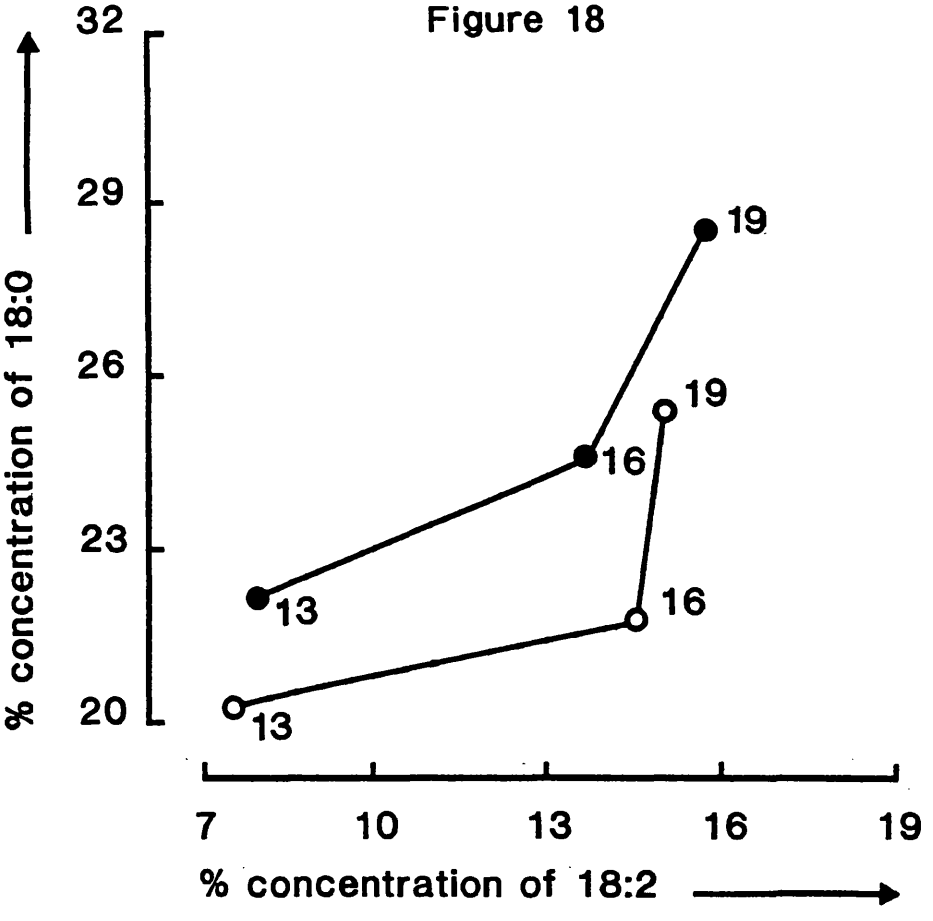
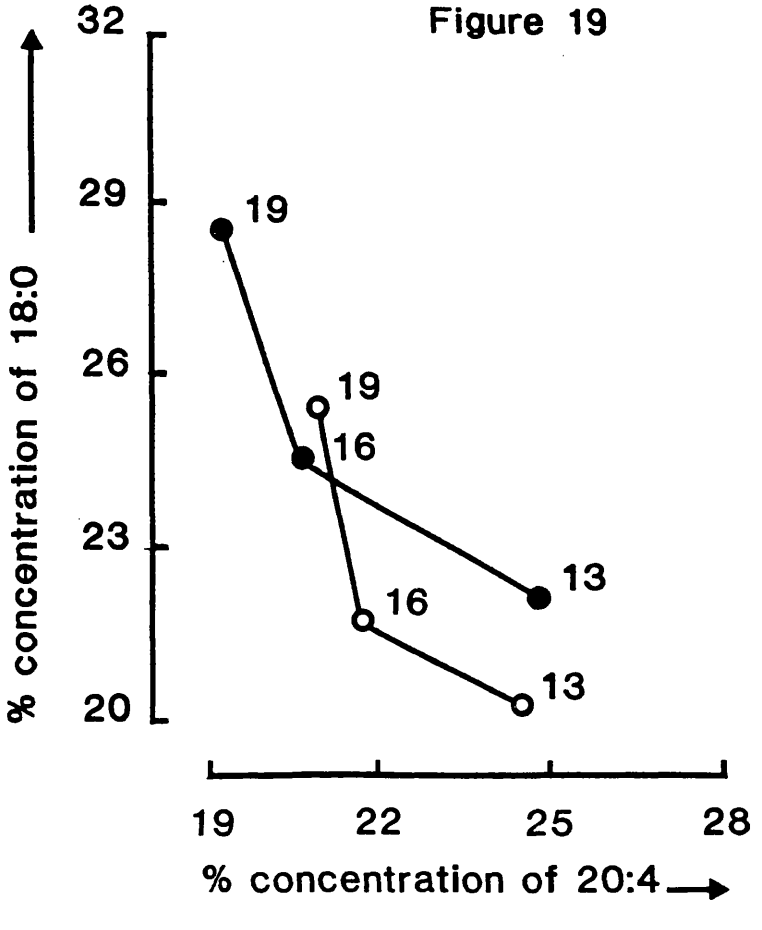


Figure 19



increase in the concentration of palmitic acid and an obvious decrease in the concentration of stearic acid, particularly between days 1 and 6 post-hatch (Table 33; Figure 20). There were curvilinear relationships between oleic and linoleic acids (Figure 21), oleic and arachidonic acids (Figure 22) and linoleic and arachidonic acids (Figure 23); these changes were most pronounced between days 1 and 6 of the post-hatch period.

Compared with the mitochondrial phospholipids in the livers of embryos and chicks from mature parents, those from the young parents displayed, during the embryonic stages in particular, lower proportions of palmitic and stearic acids. Throughout both the embryonic and post-hatch periods, the relative concentration of arachidonic acid was higher in the livers of offspring from young parents compared with those of mature parents. The proportion of docosahexaenoic acid was also higher in the mitochondrial phospholipids of livers from embryos of young parents compared with embryos from mature parents, the situation becoming reversed during the post-hatch period.

In the phospholipid fraction of the heart mitochondria, by far the major unsaturated fatty acids present during the final week of incubation were oleic and arachidonic with, in most instances, arachidonic acid being the higher. Relative concentrations of linoleic and docosahexaenoic acids were similar to those in the phospholipids of the liver mitochondria during the embryonic period. The remaining unsaturated fatty acids represented less than 4 per cent of the total fatty acids. In contrast to that displayed by the mitochondrial phospholipids of the liver, those of the heart showed no obvious changes in the levels of palmitic and stearic acids during the final week of incubation. As in the case of the liver, there was a slight increase in the total proportion of unsaturated fatty acids within the mitochondrial phospholipids as the time of hatching approached; within the polyunsaturated fatty acids, the proportion of

Figure 20

The relationship between the concentrations (weight percentages of the total fatty acids) of palmitic (16:0) and stearic acids (18:0) in the phospholipids of the mitochondria (●) and microsomes (○) of chick livers at days 1 and 6 of post-hatch.

The results are for offspring from mature parents.

Figure 21

The relationship between the concentrations (weight percentages of the total fatty acids) of oleic (18:1) and linoleic acids (18:2) in the phospholipids of the mitochondria (●) microsomes (○) of chick livers at days 1 and 6 of post-hatch.

The results are for offspring from mature parents.

Figure 20

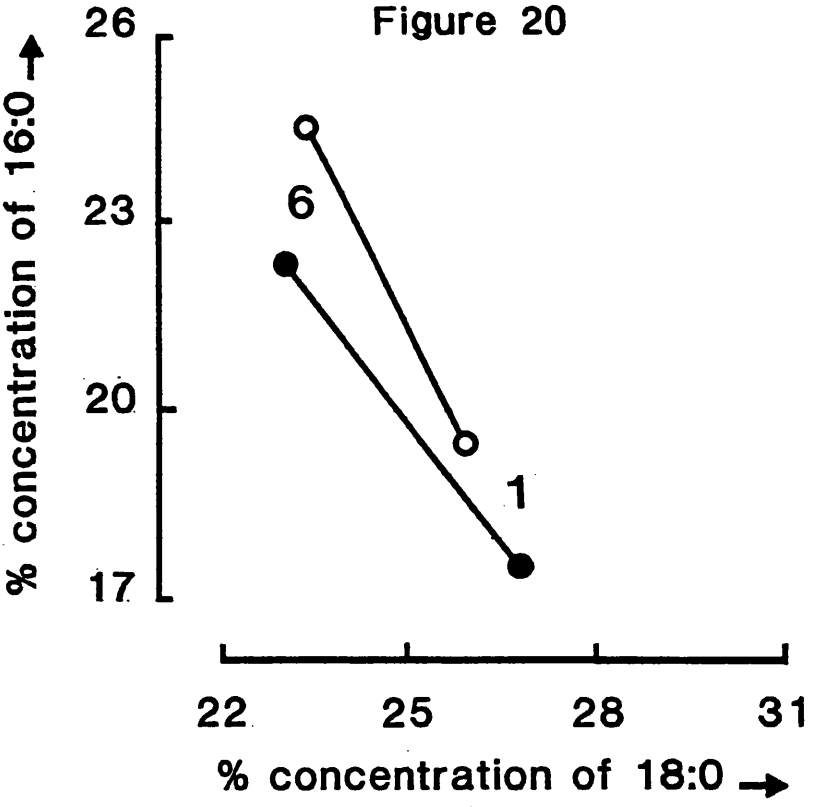


Figure 21

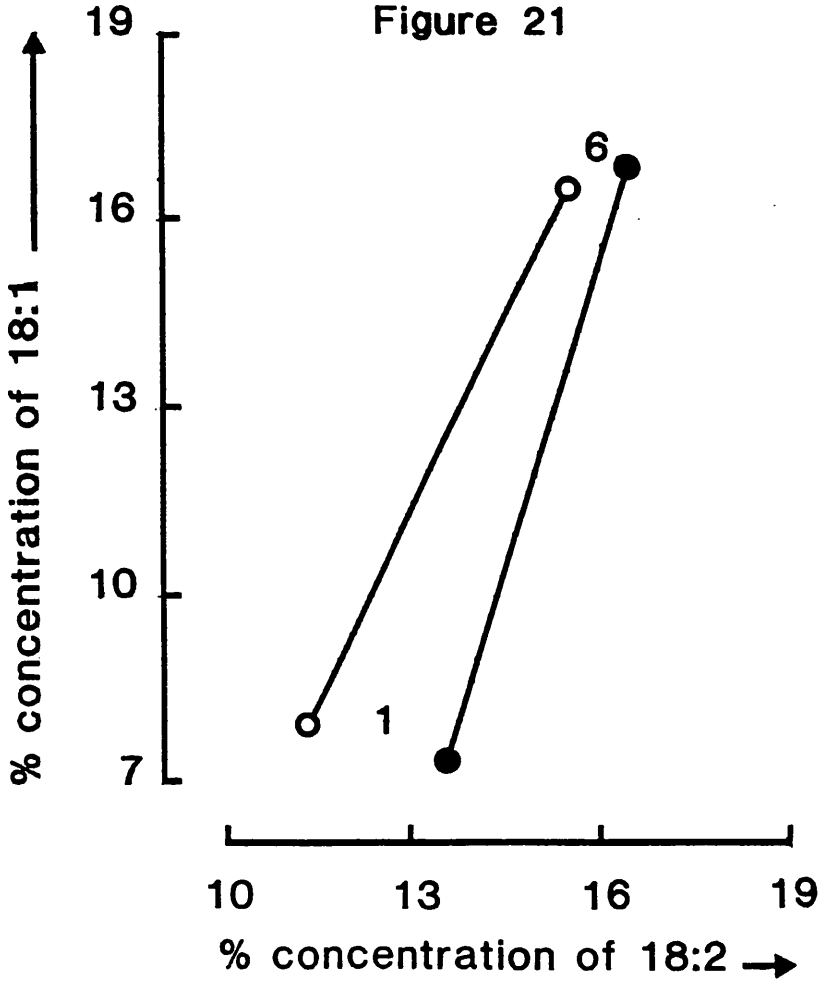


Figure 22

The relationship between the concentrations (weight percentages of the total fatty acids) of oleic (18:1) and arachidonic acids (20:4) in the phospholipids of the mitochondria (●) and microsomes (○) of chick livers at days 1 and 6 of post-hatch. The results are for offspring from mature parents.

Figure 23

The relationship between the concentrations (weight percentages of the total fatty acids) of linoleic (18:2) and arachidonic acids (20:4) in the phospholipids of the mitochondria (●) and microsomes (○) of chick livers at days 1 and 6 of post-hatch. The results are for offspring from mature parents.

Figure 22

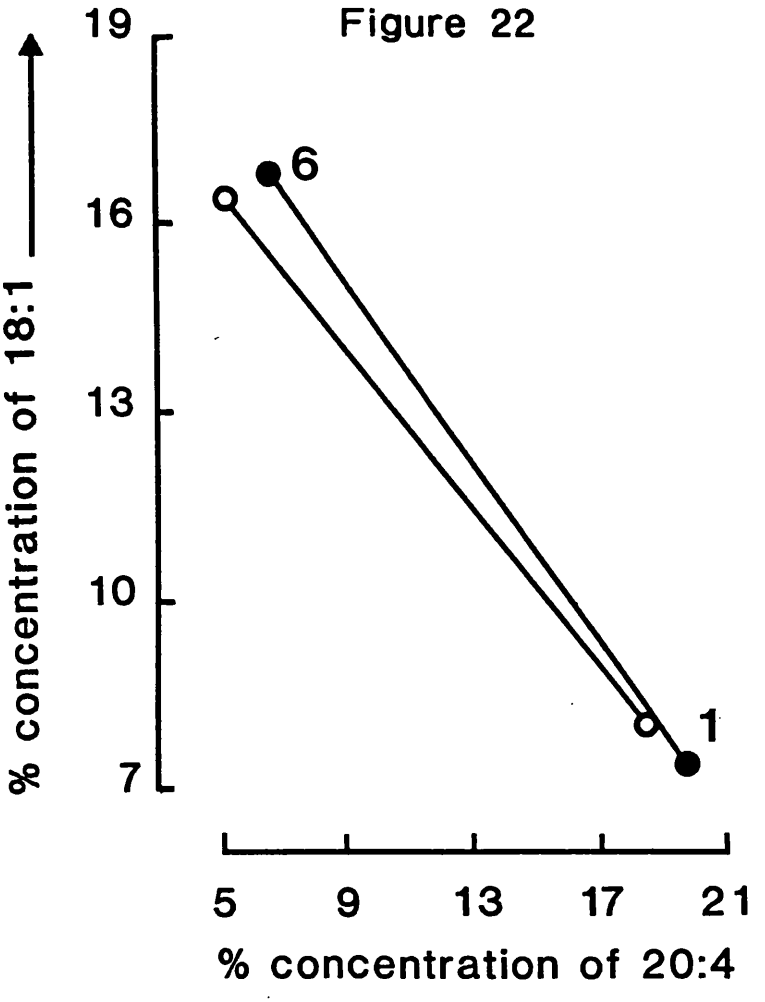
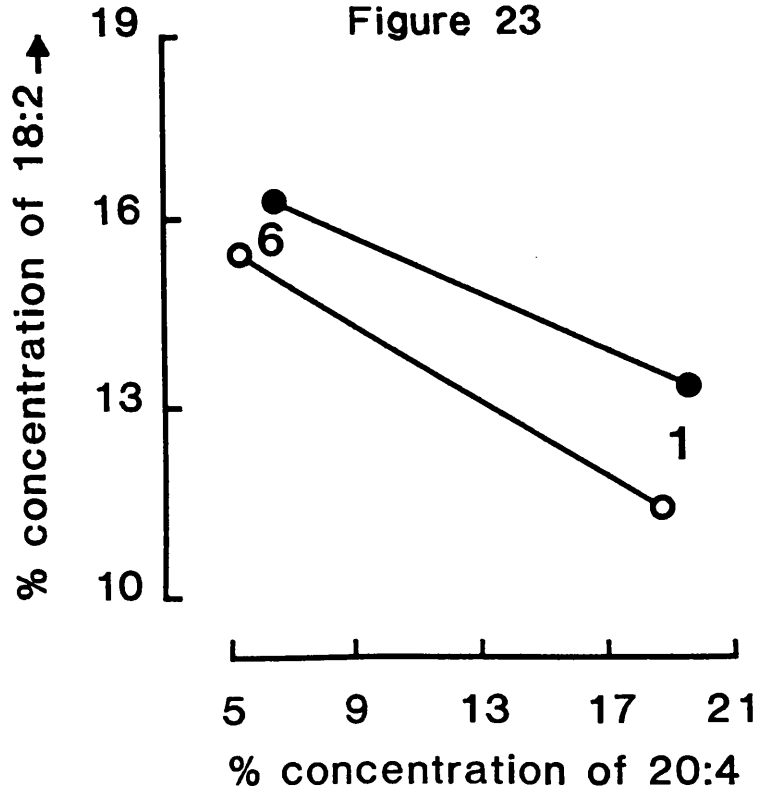


Figure 23



linoleic acid increased and that of docosahexaenoic acid decreased during the last seven days of embryonic development. In contrast to the liver, the proportion of arachidonic acid did not decrease during incubation. The changes in linoleic acid concentration, as in the case of the liver, were most pronounced between the thirteenth and sixteenth days of incubation. Pronounced decreases occurred in the concentrations of palmitic and docosahexaenoic acids between the nineteenth day of incubation and day 1 of post-hatch. Following hatching, there was a replacement of arachidonic acid by oleic and linoleic acids so that by day 12 of post-hatch, the concentration of linoleic acid was some three-fold greater than the concentration of arachidonic acid. The proportion of docosahexaenoic acid also decreased between days 1 and 12 of the post-hatch period.

In contrast to embryos from mature parents, there was a significant increase in the relative concentration of arachidonic acid in the heart organelles of embryos from young parents, prior to hatching. With the exception of palmitic acid, which was lower in the mitochondrial phospholipids of embryos from young parents, there were only minor differences in the fatty acid composition of the heart mitochondria of embryos and chicks from mature and young parents. From the above, it can be seen that, although relationships existed between the concentration changes of saturated and unsaturated fatty acids in the heart during incubation, they were far less prominent than existed for the liver.

As in the case of the liver and heart, palmitic and stearic acids together accounted for about 45 to 50 per cent of the total identifiable fatty acids in the mitochondrial phospholipids of the brain. In contrast to the liver and heart however, by far the major polyunsaturated fatty acid present throughout the embryonic and post-hatch periods was docosahexaenoic acid, but accompanied by substantial proportions of

arachidonic acid; levels of linoleic acid were exceedingly low throughout development. Compared with the liver and heart, the concentrations of palmitoleic and oleic acids within the phospholipids of the brain mitochondria were much higher. As in the case of the mitochondrial phospholipids of the liver, the relative concentration of stearic acid increased during the embryonic period. A small decrease in palmitic acid concentration during post-hatch was compensated for by increases in the proportions of stearic and oleic acids. In contrast to both the liver and heart, the brain mitochondria showed a relatively stable fatty acid composition during both the embryonic and post-hatch periods. There were no obvious differences in fatty acid compositions between embryos and chicks from mature and young parents.

7.1.2 Microsomes.

The fatty acid compositions of the microsomal phospholipids of the three tissues were very similar to that of the mitochondria. Thus, in the microsomes of the liver, as in the case of the mitochondria, there were obvious relationships between combinations of the saturated and unsaturated fatty acids (Figures 14, 15, 16, 17, 18, 19, 20, 21, 22 and 23). In general, the fatty acid patterns of the microsomal phospholipids in livers of embryos and chicks from young parents were similar to those of offspring from mature parents throughout development. The differences in the proportions of docosahexaenoic acid between the two parental groups also resembled the pattern displayed by the mitochondria, in which there were higher concentrations of docosahexaenoic acid in the phospholipids of the livers of offspring from young parents during incubation and lower concentrations during the post-hatch periods.

The microsomal phospholipids of the heart were characterised by a considerable increase in the proportion of linoleic acid following hatching and to a lesser extent, an increase in the proportion of oleic acid. Corresponding decreases occurred in the proportions of arachidonic acid, which feature was displayed by chicks from both mature and young parents. Apart from some minor differences with respect to stearic, linoleic and docosahexaenoic acids, the patterns of fatty acid changes displayed by embryos and chicks from the parental groups were very similar.

Although the microsomal phospholipids of the brain, as in the case of the mitochondria, showed very little change in fatty acid composition over the embryonic and post-hatch periods, there was an obvious reduction in the proportion of docosahexaenoic acid following hatching. The embryos and chicks from mature and young parents showed virtually identical fatty acid patterns.

7.1.3 Supernatant.

In overall terms, the fatty acid patterns and their changes in the phospholipids of the supernatant fractions of all three tissues were very similar to those displayed by the membranous components. However, as can be seen, the supernatants showed a somewhat greater variability in fatty acid composition than those of the membrane fractions. Consequently, in the liver supernatant, although relationships existed between individual saturated and polyunsaturated fatty acids, the relationships were not as clearly defined as in the mitochondria and microsomes. Although comparable changes in the proportions of the polyunsaturated fatty acids were displayed by embryos and chicks from mature and young parents, chicks from the young parents failed to show any change in the concentration of palmitic acid following hatching.

With the exception of changes in the proportions of arachidonic and linoleic acids following hatching, the phospholipids of the heart supernatant showed no appreciable changes in the fatty acid composition. Compared with embryos and chicks from mature parents, the phospholipids of the supernatant of progeny from young parents showed consistently lower proportions of docosahexaenoic acid during both the embryonic and post-hatch periods; there was also a lower proportion of linoleic acid at the time of hatching.

Within the phospholipid fraction of the brain supernatant, the only notable fatty acid changes were alterations in the proportions of palmitic acid during the embryonic period. The relative concentrations of docosahexaenoic acid were higher and oleic acid lower in the phospholipid fraction for embryos from mature parents at the time of hatching, compared with those from young parents.

Summary of section 7.1

1) Although significant differences did exist, in general there were marked similarities in fatty acid composition between the phospholipids of the mitochondria, microsomes and supernatants.

2) In all cases palmitic and stearic acids accounted for between 40 and 50 per cent of the total fatty acids in the phospholipids.

3) The relative concentrations of unsaturated fatty acids in the subcellular fractions of the liver and heart were very similar to each other. Arachidonic acid in particular, dominated the phospholipid fraction during the embryonic period; after hatch, this was replaced by linoleic and oleic acids. In contrast to the liver and heart, the subcellular fractions of the brain were characterised by very high concentrations of docosahexaenoic acid; the relative concentration of linoleic acid was exceedingly small.

4) Curvilinear relationships existed between saturated and polyunsaturated fatty acids existed in the subcellular fractions of the liver, particularly during the embryonic stage. In contrast, the fatty acid composition of the phospholipid fraction from brain subcellular isolates was relatively constant throughout development.

5) Notable differences existed between the fatty acid patterns of the embryonic and post-hatch periods.

6) Similarly, notable differences existed between the fatty acid patterns during the embryonic and post-hatch periods in progeny from mature and young parents.

7.2 Triglycerides.

The relative concentrations (expressed as mean percentages per unit weight of total fatty acids) of the major fatty acid components in the triglycerides of the mitochondria in the liver, heart and brain from embryos and chicks are given in Tables 60 61, 63 64, 66 and 67 respectively, the microsomes in Tables 69 70, 72 73, 75 and 76 and the supernatants, in Tables 78 79, 81 82, 84 and 85. The statistical significances of the changes in concentrations of the fatty acid components with increasing embryonic and post-hatch age and the differences between the embryos and chicks from mature and young parents are given in Tables 62a b, 65a b, 68a b (mitochondria); 71a b, 74a b, 77a b (microsomes); 80a b, 83a b, 86a b (supernatant).

7.2.1 Mitochondria.

The fatty acid composition of the triglyceride fraction in the liver mitochondria showed many similarities to the heart and brain mitochondria respectively. Although differences in fatty acid patterns did exist, these were most obvious during the post-hatch period only. Thus, in all cases, oleic acid was by far the major unsaturated fatty acid throughout both the embryonic and post-hatch periods. The relative concentration of oleic acid fluctuated during the final week of embryonic development but showed a marked increase at the time of hatching in the liver and heart mitochondria in particular. The main saturated fatty acid was palmitic; there was an obvious fluctuation in the concentration of palmitic acid during the embryonic period which showed significance only at $P < 0.05$. By comparison, levels of stearic acid were very much lower throughout, but the fluctuations displayed were not significant. In general the changes in concentrations of palmitic and stearic acids parallelled each other to an extent that, together, a constant degree of saturation was maintained.

The relative concentration of linoleic acid in the triglyceride fraction of the liver mitochondria in particular, was somewhat lower than that displayed by the phospholipid fraction; by contrast, the relative concentration of linoleic acid in the triglyceride fraction of the brain mitochondria was generally higher than that displayed by the phospholipids (see Sections 7.1.1 and 7.2.1). All the tissues displayed a relatively constant proportion of linoleic acid during the embryonic period; at hatching though, the concentration was considerably increased. Arachidonic acid constituted a relatively small percentage of the triglyceride fraction of all the tissues; in the liver mitochondria, the levels of arachidonic acid decreased during the final week of incubation.

A major feature of the mitochondrial triglycerides of the liver was an unusually high proportion of docosahexaenoic acid (often greater than 10 per cent during the embryonic period). Concentrations of docosahexaenoic acid are clearly shown to have exceeded those of the heart and brain. Indeed, during the embryonic period, the concentration of docosahexaenoic acid in the triglyceride fraction of the liver mitochondria was in general, much greater than in the phospholipids, with which the fatty acid is usually associated. By comparison, concentrations of docosahexaenoic acid in the phospholipids of the heart and brain mitochondria were higher than in the triglycerides (see Sections 7.1.1 and 7.2.1). During the embryonic period, all three tissues underwent a decrease in relative concentrations of docosahexaenoic acid within the mitochondrial triglycerides.

Following hatching, the triglycerides of both the liver and brain mitochondria showed an increase in their extent of saturation. In the liver, the relative concentrations of all polyunsaturated fatty acids decreased with compensatory increases in both the saturated and monounsaturated fatty acids, but in particular oleic acid. In the

triglycerides of the brain mitochondria, as in the case of the liver, there was an obvious decrease in the proportion of linoleic acid during the latter part of the post-hatch period. In the heart, the most notable change in fatty acid composition was confined to the increase in palmitoleic acid. A feature of all three tissue mitochondria, but in particular the liver, was the decrease in docosahexaenoic acid levels following hatching.

In comparison with progeny from mature parents, mitochondria from the livers of progeny from young parents showed lower concentrations of palmitic acid throughout the embryonic and post-hatch periods. At the later stages of the post-hatch period there were lower concentrations also of stearic acid. Comparisons of heart mitochondrial triglycerides revealed minor differences only. More obvious differences however, were displayed by the brain mitochondrial triglycerides between progeny from the mature and young parents, both in the relative concentrations and the relative changes in fatty acids. Changes in fatty acid concentrations displayed between the progeny from the two parental groups were most obvious with respect to palmitic and stearic acids. Thus, there was an appreciable decrease in the relative concentration of palmitic acid, particularly during the latter part of the post-hatch period in progeny from young parents. Conversely, levels of stearic acid in the brain mitochondria showed no obvious changes in chicks from young parents, whereas they increased in chicks from mature parents. Differences were also displayed between the chicks from the two parental groups in the relative concentrations of oleic and linoleic acids during the post-hatch periods.

7.2.2 Microsomes.

In general terms, the fatty acid composition and changes in the triglyceride fraction of the microsomes during embryonic and post-hatch development were similar to those observed in the mitochondria for all three tissues. Compared with the mitochondria however, slight differences did exist. As in the case of the mitochondria, the relative concentration of docosahexaenoic acid in the triglyceride fraction of the liver microsomes was very high and exceeded that displayed by the phospholipids during the embryonic period (see Sections 7.1.2 and 7.2.2); there was also a rapid decline in the concentration of docosahexaenoic acid immediately post-hatch. In contrast to embryos from mature parents, the microsomal triglyceride fraction from livers of embryos from young parents showed a relatively constant proportion of oleic acid during the final week of incubation. The relative concentration of stearic acid was higher during the embryonic period in offspring from young parents compared with those from mature parents; the pattern was reversed following hatching.

In comparison with the heart mitochondrial triglycerides, those of the microsomes displayed a more obvious decrease in the proportion of palmitoleic acid following hatching. The relative concentration of palmitoleic acid was also affected by age and thus, in progeny from mature parents, the concentration of palmitoleic acid in the mitochondrial triglycerides of the heart was higher during the embryonic period.

By comparison with the mitochondrial fraction of the brain, the relative concentration of oleic acid in the microsomal triglycerides increased during the incubation period. Several differences existed in the composition of the brain microsomal triglycerides between progeny from the two parental groups. Whereas by the end of the post-hatch period concentrations of palmitic and oleic acids were similar between the two

parental groups, there were differences at the time of hatching. Immediately following hatching, differences in the concentrations of linoleic acid between the two parental groups were apparent. In overall terms however, the fatty acid changes which occurred within the microsomal triglycerides of the brain were similar to those which had been observed for the mitochondria.

7.2.3 Supernatant.

In general the triglycerides of the supernatant fraction from all three tissues exhibited similar compositions and patterns of change in their fatty acids to the mitochondria and microsomes, with for instance, unusually high concentrations of docosahexaenoic acid in the liver during the incubation period, which rapidly reduced following hatching. Some slight differences, however, were observed in both the embryonic and post-hatch periods between the membrane and supernatant fractions. In general, during embryonic development, the concentrations of docosahexaenoic acid were lower and those of linoleic and oleic acids higher in the liver supernatant, than those observed in the mitochondria and microsomes. In the supernatant triglycerides for example, there were large fluctuations in the relative concentrations of palmitic acid during the post-hatch period compared with the membranous components.

As in the case of the microsomes, the triglyceride fraction in the liver supernatants of progeny from mature parents displayed a lower concentration of stearic acid during the final week of incubation compared with those from young parents. At the time of hatching and during the early stages of the post-hatch periods, the relative concentrations of linoleic acid were lower in the liver supernatant of progeny from young parents compared with those from mature parents.

In the triglycerides of the heart supernatant, as in the case of the liver, the relative concentration of linoleic acid was lower in embryos from young parents at the time of hatching compared with those from mature parents. Other differences between the two parental groups were minor and lacked consistency in pattern throughout both the embryonic and post-hatch periods.

In contrast to the mitochondria and the microsomes, the triglycerides of the brain supernatant displayed a relatively constant proportion of oleic acid. Compared with embryos from mature parents, those from young parents displayed, within the supernatant triglycerides, lower levels of docosahexaenoic acid at day 13 of incubation; concentrations increased however, to reach a higher level at the time of hatching, than that displayed by embryos from the mature group. Overall, the relative concentrations of palmitic acid were higher in the triglycerides of the brain supernatant from the young group during the embryonic period compared with the mature group.

Summary of section 7.2

1) Although significant differences did exist, in overall terms there were marked similarities in fatty acid composition between the triglycerides of the mitochondria, microsomes and supernatants.

2) In all cases palmitic and oleic acids accounted for between 60 and 75 per cent of the total fatty acids in the triglycerides.

3) Both incubation and post-hatch periods were associated with marked changes in fatty acid composition.

4) In the subcellular components of the liver, an unusual feature of the triglyceride fraction was the high concentration of docosahexaenoic acid during the embryonic period. This concentration frequently exceeded 10 per cent compared with approximately 6 per cent identified in the phospholipids. Differences in the concentration of docosahexaenoic acid between the phospholipids and triglycerides were not observed to the same extent in the subcellular fractions of the heart and brain.

5) Differences in fatty acid composition of all three tissues were observed between progeny from mature and young parents; these differences however, were often minor and lacked consistency throughout both the embryonic and post-hatch periods.

7.3 Cholesteryl esters.

The relative concentrations (expressed as mean percentages per unit weight of total fatty acids) of the major fatty acids in the cholesteryl esters of the mitochondria in the liver, heart and brain from embryos and chicks are given in Tables 87 88, 90 91, 93 and 94 respectively, the microsomes in Tables 96 97, 99 100, 102 and 103 and the supernatants, in Tables 105 106, 108 109, 111 and 112. The statistical significance of the changes in concentrations of the fatty acids components in the cholesterol ester fraction with increasing embryonic and post-hatch age and the differences between the embryos and chicks from mature and young parents, are given in Tables 89a b, 92a b, 95a b (mitochondria); 98a b, 101a b, 104a b (microsomes); 107a b, 110a b, 113a b (supernatant).

7.3.1 Mitochondria.

By far the most prominent feature of the cholesterol ester fraction within the mitochondria was that displayed by the liver. In the liver, oleic acid accounted for between 60 and 70 per cent of the total fatty acids during the embryonic period. This unusually high level of oleic acid was maintained during the early post-hatch period, but showed a significant decrease by day 12 of post-hatch. Apart from palmitic and linoleic acids, the relative concentrations of all other fatty acids were, by comparison, lower throughout incubation and the early post-hatch period. A feature of the cholesterol ester fractions however, was the elevated level of palmitoleic acid during the embryonic period, its concentration being significantly higher than that displayed within the phospholipids of the liver mitochondria (see Section 7.1.1). The decrease in the relative concentration of oleic acid observed at day 12 of post-hatch was commensurate with increases in the concentrations of palmitic and stearic acids.

Although somewhat lower than the liver, the heart mitochondria also showed a predominance of oleic acid throughout the incubation and early post-hatch periods; as in the case of the liver, concomitant changes in the concentrations of palmitic and stearic acids were observed at post-hatch. Levels of oleic acid in the brain were distinctly lower than those displayed in particular by the liver. However, with the exception of linoleic acid, fatty acid patterns of change during the incubation and post-hatch periods were similar to those displayed by the liver and heart mitochondria; in the case of linoleic acid, there was an obvious decrease in its concentration with time after hatch. Low concentrations only of C20 and C22 polyunsaturated fatty acids were displayed by the cholesteryl esters of all tissue mitochondria.

The fatty acid distributions and their changes in the mitochondria obtained from embryos and chicks from young parents showed marked similarities to those from mature parents; the small differences shown, lacked consistency.

7.3.2 Microsomes.

In overall terms the fatty acid patterns displayed by the microsomes were very similar to the fatty acid patterns displayed by the cholesteryl esters of the mitochondria. Thus, high concentrations of oleic acid were present throughout incubation and early post-hatch showing a decrease at day 12, compensatory increases in palmitic and stearic acids, lower concentrations of oleic acid in both the heart and brain microsomes etc., were also all present. However, the changes in fatty acid compositions were in most instances, but especially within the liver, more obvious when compared with the changes that were observed to occur within the cholesteryl esters of the mitochondria. In contrast to the mitochondria where there were no differences in fatty acid composition of the tissues between offspring from mature and young parents, in the case of the microsomes, the heart showed a selection of quite distinct differences.

Thus, levels of palmitic acid within the heart microsomes of embryos from young parents showed significantly lower concentrations of palmitic acid and higher concentrations of oleic acid; such differences were not maintained following hatching. As in the case of the mitochondrial cholesteryl esters, any differences in the fatty acid compositions of the cholesteryl esters of the liver and brain microsomes between mature and young parents were minor and inconsistent.

7.3.3 Supernatant.

The overall compositions and changes in fatty acids of the cholesteryl esters within the supernatant fractions were also similar to those displayed by the mitochondria and microsomes. As in the case of the liver supernatant, however, the relative concentrations of oleic acid were consistently higher than within either the mitochondria or microsomes and the relative concentrations of palmitic acid were particularly low. In general, the supernatant cholesteryl esters of all three tissues showed significantly higher concentrations of linoleic acid both in the embryonic and post-hatch periods. As in the case of both the mitochondria and microsomes, only minor changes were displayed by heart and brain supernatants between the embryos and chicks from mature and young parents; the exception to this was a difference in linoleic acid levels within the heart following hatching. In the case of the brain supernatant, however, the concentration of oleic acid was consistently higher in embryos from young parents.

Summary of section 7.3

1) The fatty acid distributions during both embryonic and post-hatch periods for the cholesteryl esters of all three tissue fractions showed a marked similarity to each other.

2) The major feature of the supernatant fraction was the particularly high level of oleic acid associated with the liver in particular and the heart.

3) Whereas fatty acid compositions showed consistency during the embryonic period, more marked changes occurred during the late post-hatch period involving compensatory changes between palmitic, oleic, linoleic etc.

4) The relative concentrations of C20 and C22 polyunsaturated fatty acids were extremely low throughout the tissue fractions.

5) With some minor exceptions, for example, levels of oleic acid in the brain supernatant, the cholesteryl esters from all tissue fractions showed a marked similarity in fatty acid composition between embryos and chicks from mature and young parents.

8 The distribution of the major fatty acid moieties between the mitochondria, microsomes and cellular supernatant in the liver, heart and brain.

As can be expected, the distribution of the fatty acids between the subcellular fractions were in accordance with the overall lipid distribution (see Section 6). This is illustrated in Tables 114 and 115 with respect to the distribution of the phospholipid fatty acids within the liver fractions. Thus, as can be seen, in progeny from mature parents, all the fatty acids were specifically and consistently associated with the membranous components during the embryonic and post-hatch periods. During the embryonic period, there was a predominant association with the microsomes, whereas following hatching, the association was mainly with the mitochondria. Although the embryos and chicks from young parents also displayed a preferential association of fatty acids with the membranous components, there was a marked difference in their distributions between the mitochondria and microsomes. In contrast to the embryos from mature parents, those from young parents thus showed a preferential association of fatty acids with the mitochondria, whilst in the chicks, the disparity in fatty acid distribution between the mitochondria and microsomes was increased even further.

In comparison with the phospholipids, the triglyceride fatty acids showed a far more even distribution between the three subcellular fractions during both embryonic and post-hatch development in progeny from mature and young parents. The cholesterol ester fatty acids were heavily biased towards the supernatant fraction during embryonic development, becoming more evenly distributed between the fractions following hatching.

In the case of the phospholipids, a major feature was the specific association of C20 and C22 polyunsaturated fatty acids with the membrane fractions. A feature of the triglycerides was the specific association of docosahexaenoic acid in the membrane fractions during the embryonic period. In the case of the cholesteryl esters, the distribution of the fatty acids was such that the supernatant showed very high proportions of oleic acid.

9 Desmosterol: cholesterol ratios in the liver and the brain.

In view of the extensive accumulations of cholesterol within the tissues of the embryo during incubation (see Section 5), it was deemed worthwhile to investigate whether tissue concentrations were solely accountable through the absorption of preformed cholesterol from the yolk, or whether *de novo* synthesis within the tissues was involved. As an indication of the possible involvement of *de novo* synthesis, changing ratios of desmosterol: cholesterol were determined in embryos and chicks from normal parent stock in the liver and brain, that is, those tissues in which concentrations of free cholesterol were the most prominent.

Table 116a shows the desmosterol: cholesterol ratios in the brain organelles of embryos and chicks from mature and young parents. Whereas meaningful ratios of desmosterol: cholesterol were obtainable throughout for the brain, levels of desmosterol in the liver were so small as to render the ratios meaningless. In the case of the liver therefore, it is implied that cholesterol accumulations observed during the embryonic period arise almost exclusively from the yolk lipid uptake. By contrast, in the brain, a significant amount of cholesterol, especially during the period prior to extensive yolk lipid uptake, arises from *de novo* synthesis; as can be seen in embryos and chicks from mature parents, the ratio of desmosterol: cholesterol at days 13 and

16 of incubation consistently exceeded the ratios just prior to and post-hatching (Tables 116a b, 117a b). By contrast, in embryos from young parents, the desmosterol: cholesterol ratios did not decrease during the incubation period. In accordance with the absolute weights of lipid noted in Section 3.3, the absolute levels of desmosterol and cholesterol were much higher in the brain mitochondria of offspring from young parents compared with those from mature parents.

10 Relationship between alpha tocopherol and tissue levels of polyunsaturated fatty acids in embryos and chicks.

A feature of the studies has been the considerable increase in the level of polyunsaturated fatty acids that occurs in the yolk sac membrane and tissues of the chick embryo during the final week of incubation, compared with the polyunsaturated fatty acid levels of the chick embryo in the yolk as laid. This is illustrated in Table 118 (see also Sections 6 and 9 of the Literature Review). The high concentrations of polyunsaturated fatty acids within the tissue lipids enhances their susceptibility to peroxidation and the formation of hydroperoxides, free radicals and other toxic products, all of which are detrimental to cell function (Ohki and Goldup, 1968; Antonov *et al.*, 1976; Bulkley, 1983; Marx, 1987). It is therefore of paramount importance that adequate protection is provided to prevent oxidation. In natural systems, it has been established that vitamin E has a major role as a biological antioxidant and free radical scavenger (see Tappel, 1972; Hoeskra, 1975; see McCay and King, 1980).

An investigation into the relative proportional changes of vitamin E (in this case α-tocopherol) and the polyunsaturated fatty acids of the embryonic and post-hatch tissues will provide data on both the overall protective ability afforded to the cell and possible interactions at the subcellular level between the membrane lipids and their function.

The following concerns:

1) the development of a suitable method for the quantification of α-tocopherol levels within the lipid enriched tissues of the embryos and chicks from mature and young parents;

2) observations on comparative levels of α-tocopherol and polyunsaturated fatty acids in the yolk complex and embryonic/post-hatch tissues.

10.1 Development and methodology.

10.1.1 Sampling yolk contents.

To obtain a sample of yolk contents, free from contamination with amniotic fluid, plasma and other associated materials during incubation, proved more difficult than originally envisaged. A range of methods was tried. The use of glass syringes and other devices to aliquot the yolk contents following careful entry through the yolk sac membrane led to extensive contamination from membranous material and vitelline fluids. Attempts to maintain patency of the yolk sac membrane to secure entry into the yolk contents also failed. A simplistic method involving careful incision following blotting was finally considered to be the best that could be adopted. It was accepted that trace contamination with plasma and vitelline fluid were unavoidable. The same procedure was adopted in chicks; at this stage, error due to contamination was less, but error due to small sample size was increased.

The inhomogeneity of avian yolks arising from the cyclical rates of deposition are well known (Bellairs, 1961; see Gilbert, 1971; see Griminger, 1976). As a result of this, it was necessary to try and achieve suitable homogeneity of the aliquot. Again, a range of methods was attempted to achieve this and the most effective method proved to be the simplest, namely manual mixing of undiluted yolk with a glass rod.

10.1.2 Alpha tocopherol quantification.

Direct application of methods specified for use with serum plasma gave rise to a whole series of problems, starting with inadequate solubility in solvent and ending with a host of unidentifiable peaks on the chromatographic separation. It was apparent that the extremely high level of lipid in the samples was the source of considerable analytical problems and required extensive modification to any standard analytical quantifying procedure adopted. It was identified that separation of α-tocopherol from contaminating lipids was essential. Although separation of α-tocopherol from lipid contaminants using thin layer chromatography and colorimetry has been shown to be successful (Whittle and Pennock, 1967; Hans, De Bevere and De Leenheer, 1985), the level of lipid contamination in the present circumstances was so high as to preclude its use. Visualisation by ultra violet absorbent dyes, iodine and other recommended agents further complicated HPLC peak identification.

Throughout the analysis, minor modifications to individual steps of the procedure (see Section 7 of the Materials and Methods) had to be introduced to maximise recovery.

Whereas initial recoveries of α-tocopherol, as measured by standard spiking, were only 50-60 per cent, the method finally adopted was able to achieve consistent recoveries in excess of 95 per cent. The major factor in achieving high recoveries was

the suitable adjustment in the polarity of the extracting solvent and prevention of α-tocopherol oxidation by inorganic multivalent ions, for example cuprous and ferric ions (Emmerie and Engel, 1943; Cort, Mergens and Greene, 1978). Formation of a complex and subsequent breakdown of α-tocopherol was prevented by addition of a dilute acid solution.

In accordance with the normal quantifying procedure routinely used, it was necessary to add a suitable extract antioxidant whilst awaiting sampling.

As stated in the methodology (see Section 8 of the Materials and Methods), the shortcomings of electronic quantification adopted were overcome by suitable adjustment of the calibration curve by single point determination using a batch recalculation program on the computer.

10.2 Comparative levels of alpha tocopherol and polyunsaturated fatty acids in the tissues.

The concentrations (micrograms per gram of tissue) of α-tocopherol in the yolk, yolk sac membrane, liver, heart and brain of embryos and chicks from mature and young parents, are given in Tables 119a b. The statistical significance of the changes in concentrations of α-tocopherol with increasing embryonic and post-hatch age and the differences between progeny from mature and young parents are given in Tables 120 (yolk), 121(yolk sac membrane), 122 (liver), 123 (heart), 124 (brain), 125 (all tissues) and 126 (gall bladder and intestinal tract).

10.2.1 Yolk complex.

As can be seen, α-tocopherol remained specifically and consistently associated with the yolk contents up to day 13 of incubation. Towards the latter stages of embryonic development (that is, from day 16 to day 21), there was a reduction in the concentration of α-tocopherol in the yolk contents. As noted previously, the somewhat larger standard error observed at days 7 and 13 of incubation for α-tocopherol levels in the yolk contents was accounted for by sampling difficulties (see Section 10.1). Compared with the yolk from embryos and chicks from mature parents, those from young parents displayed lower levels of α-tocopherol per gram of yolk throughout the embryonic and post-hatch periods.

In the yolk sac membrane there was a very large and consistent increase in the level of α-tocopherol per gram of tissue from the seventh day of embryonic development until and including the first day after hatch. During the early post-hatch period, there was an appreciable reduction in α-tocopherol levels within the membrane. In the embryos and chicks from young parents, the yolk sac membrane showed much lower levels of α-tocopherol throughout both periods compared with embryos and chicks from mature parents.

10.2.2 Tissues.

The liver, like that of the yolk sac membrane, showed a marked increase in the level of α-tocopherol during the final week of incubation. There was an appreciable increase in the level of α-tocopherol per gram of liver tissue at the time of hatching, with the levels at day 1 of post-hatch being almost three fold higher than that observed immediately before hatching. Levels of α-tocopherol at day 13 of incubation were extremely low. During the early post-hatch period, there was a marked reduction in

the levels of α-tocopherol, the levels falling from more than 750 μg/g tissue to about 10 μg/g tissue at day 12 of post-hatch. These low levels of α-tocopherol were then maintained well into development. There were no obvious differences in the levels of α-tocopherol between the livers of embryos and chicks from mature and young parents.

As can be seen, there were no obvious changes in the levels of α-tocopherol per gram of heart or brain tissue throughout the embryonic and post-hatch periods. In both the heart and brain, the levels of α-tocopherol were extremely low, considerably lower than those levels observed in the liver and in some instances fell below the accurate detection threshold. There were no major differences in the levels of α-tocopherol in the heart and brain tissues between embryos and chicks from mature and young parents.

Preliminary studies on α-tocopherol levels in the gall bladder and intestinal tract during the first six days of the post-hatch period are shown in Table 126. Compared with other tissues analysed, levels of α-tocopherol were low in both. In view of the low sample weights, the changes in α-tocopherol levels in the gall bladder were difficult to interpret. Although α-tocopherol was detected in various regions of the intestinal tract, there was no consistent pattern to suggest the intestinal tract as a likely means for the destruction and subsequent removal of α-tocopherol from the tissues.

10.3 The relationship between alpha tocopherol and polyunsaturated fatty acid levels in the tissues.

The adequacy of α-tocopherol with respect to its metabolic protective role in tissue metabolism can only be obtained by direct comparisons between α-tocopherol and polyunsaturated fatty acid tissue concentrations; in particular, the latter must include

some measure to allow for the number of double bonds per molecule of fatty acid. Tables 118, 119b and Figure 24 show such a comparison for the yolk, yolk sac membrane and liver observed for the chick embryo.

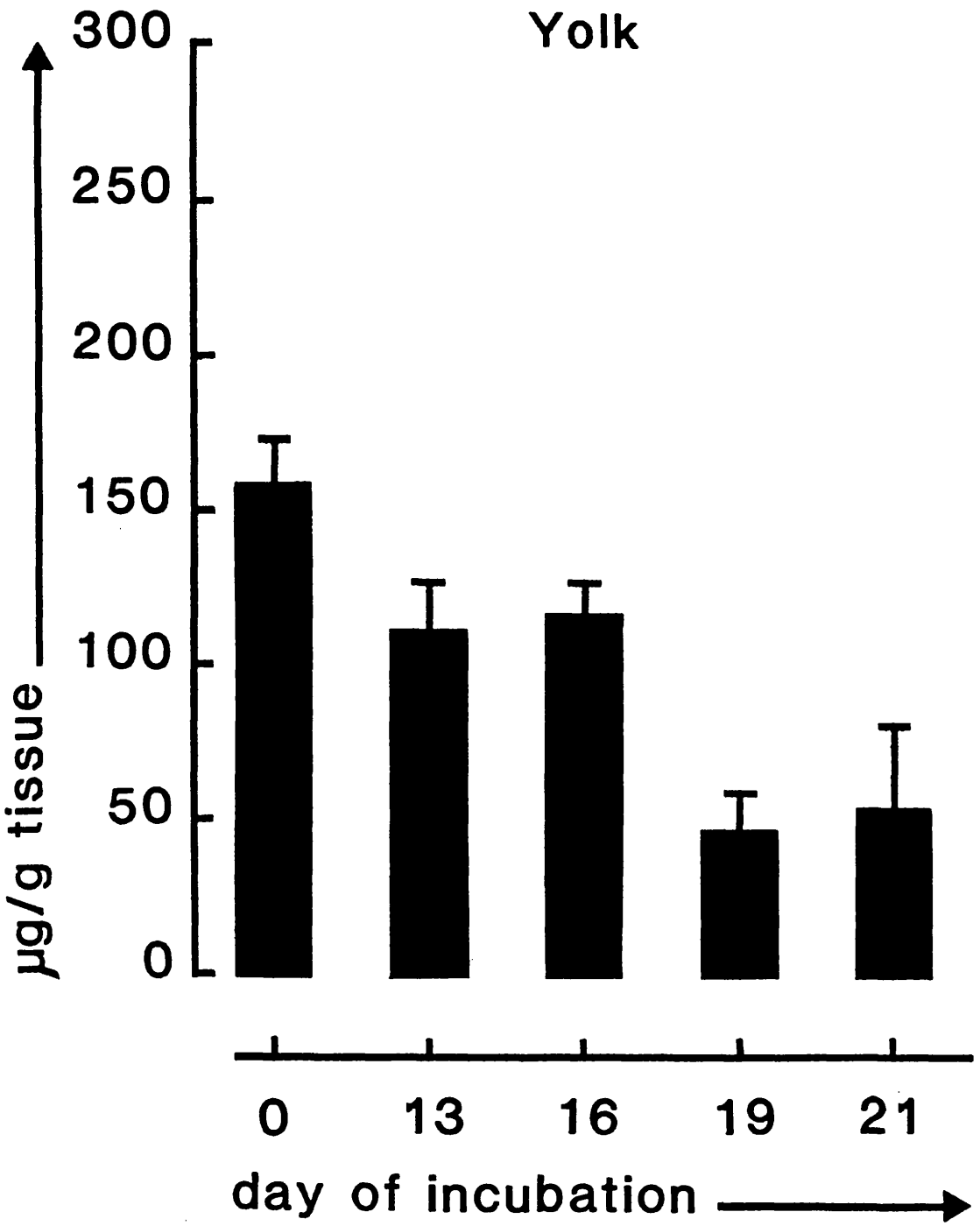
As can be seen (see Section 4.3 of the Literature Review; Section 7.1 of the Results), the extensive accumulation of lipid within the liver is associated with an accumulation of polyunsaturated fatty acids compared with the yolk contents. If account is taken of the predominance of C20 and C22 polyunsaturated fatty acids within the liver, then at day 15 of incubation, the number of double bonds present per unit weight of lipid is some three fold greater than that present within the yolk (Table 119b). The extensive accumulation of cholesteryl esters within the liver just prior to hatching (see Section 4.3 of the Literature Review and Section 5 of the Results) reduces considerably the state of unsaturation just prior to hatching. From the results shown in Table 118 and Figure 24 it is clear that whereas there is an extensive accumulation of α-tocopherol within the liver, this occurs well after a time when the state of lipid unsaturation and requirements for protection against oxidation are most extreme. At the particular time of high unsaturation, namely at day 15, levels of α-tocopherol are minimal.

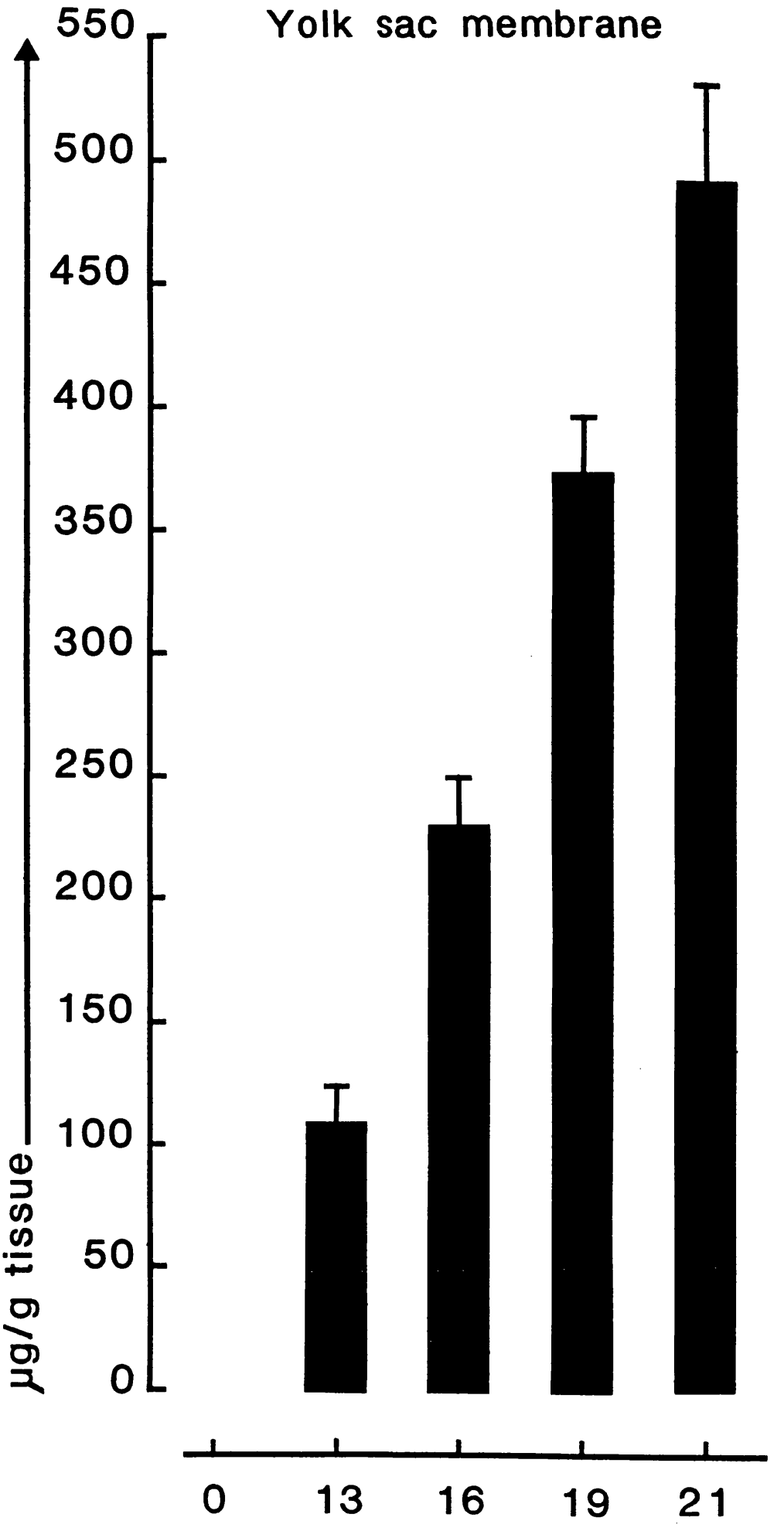
Analysis of the brain tissue from embryos and chicks showed, that in spite of extremely high levels of polyunsaturated fatty acids, in particular docosahexaenoic acid (six double bonds per molecule), levels of α-tocopherol per unit weight of tissue never exceeded approximately 5 μg/g tissue.

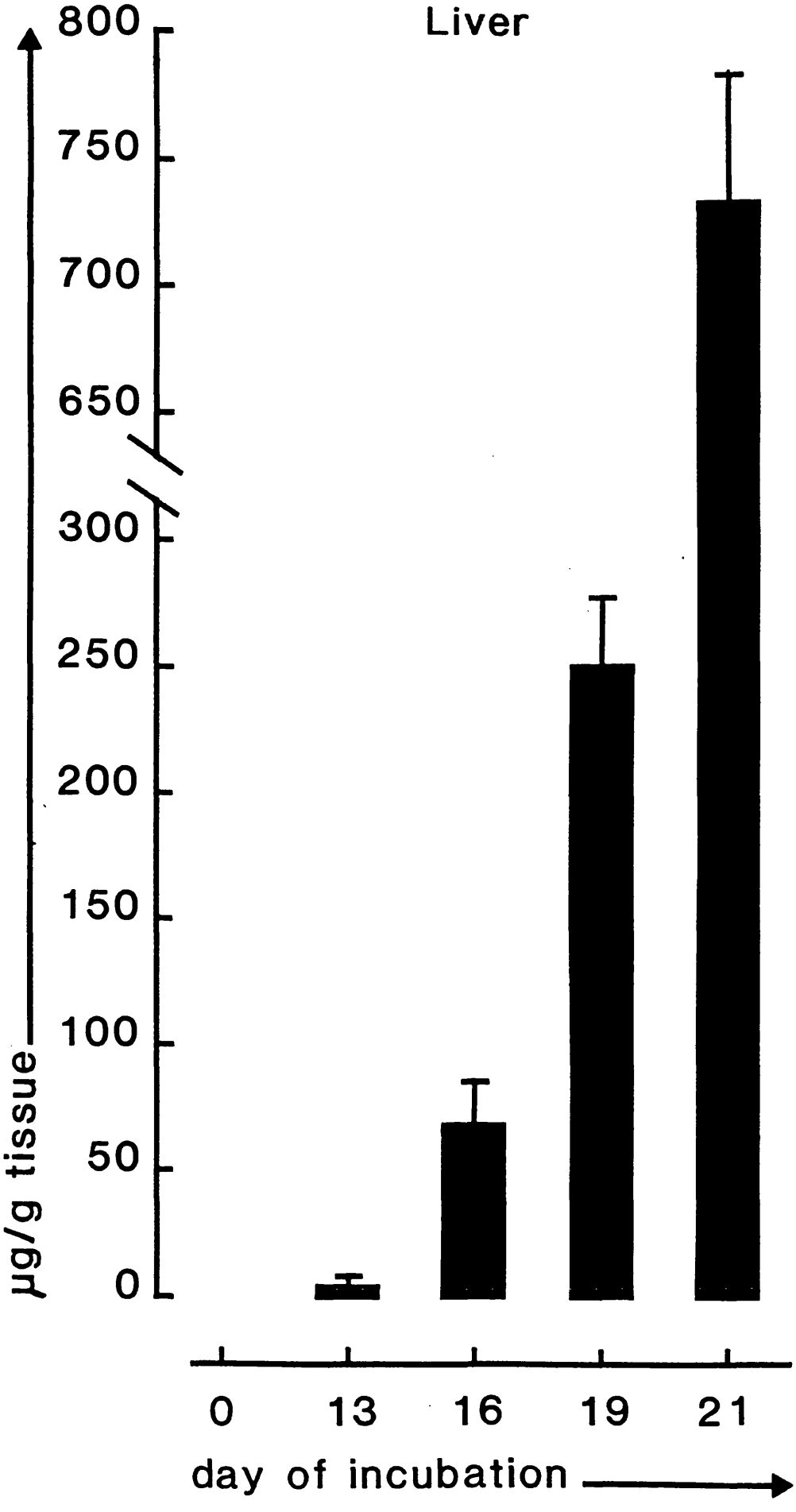
Figure 24

The concentrations (micrograms per gram of wet tissue) of alpha tocopherol in the yolk, yolk sac membrane and liver during the final week of incubation. The results are for embryos from mature parents.

Figure 24


Yolk
300
250
200
150
100
50
0
µg/g tissue
0
13
16
19
21
day of incubation


Yolk sac membrane
550
500
450
400
350
300
250
200
150
100
50
0
µg/g tissue
0
13
16
19
21


Liver
800
750
700
650
300
250
200
150
100
50
0
µg/g tissue
0
13
16
19
21
day of incubation

CHAPTER 5
DISCUSSION

1 General Introduction.

Previous studies have identified that the yolk lipid constitutes a major nutrient source for embryonic development of the chick (Noble and Moore, 1964, 1966, 1967a; Noble *et al.*, 1986a) and for a short period following hatching (Entenman *et al.*, 1940; see Romanoff, 1960; see Freeman and Vince, 1974; Noble and Ogunyemi, 1989). The same situation exists also in non avian egg laying species (Noble, Deeming, Fergusson and McCartney, 1990, 1991). In all cases the lipid not only supplies the majority of the energy for development, but also more specific components required for tissue function (Noble and Moore, 1964, 1966, 1967b,c; see Romanoff, 1960; Wood, 1974; Nakagawa *et al.*, 1982).

As in all animal species, the power house of lipid metabolism is the liver (Goldman, Chaikoff *et al.*, 1950; Ranney *et al.*, 1951; see Noble and Cocchi, 1991). Consequently, in the chick embryo, the heavy emphasis on lipid metabolism is associated with extensive lipid metabolic and compositional changes in the liver (Moore and Doran, 1962; Noble and Moore, 1964, 1966, 1967a; Wood, 1974). The limited observations presently made on the liver lipid changes during embryonic development concur with previous observations involving extensive accumulations as measured both biochemically and observed microscopically (Moore and Doran, 1962; Noble and Moore, 1964, 1966, 1967a; Wood, 1974; Noble and Yafei, 1988; Yafei and Noble, 1990). As has been shown previously (Noble and Ogunyemi, 1988, 1989; Table 5 of the Results Section), although following hatching the total amounts of lipid associated with the whole liver continued to increase, lipid accumulation per unit weight of tissue decreased.

Morphologically, the major feature of the liver during embryonic development was the vast cytosolic accumulation of discrete lipid droplets. Previous electron microscopy studies have identified similar droplet accumulation (Noble, *et al.*, 1988; Noble and Yafei, 1988; Yafei and Noble, 1990) but these studies were solely confined to the embryonic period. It was apparent from the present observations (plates not shown in the Results section) that following hatching, the extent of cytosolic lipid accumulation was considerably reduced; these changes were not observable immediately after hatching, but became obvious after several days.

From previous analytical investigations it has been shown that extensive lipid compositional changes occur immediately following hatching and in particular, they involve reductions in the levels of specific components that have accumulated during the embryonic period, for example, cholesteryl esters (Noble and Ogunyemi, 1988, 1989). The rapid reduction in cholesterol ester concentration must be associated with distinct alterations to the biochemical conformation of the lipoprotein complexes present. It may be suggested therefore, that there were morphological adjustments in response to changes in the role of the lipoprotein complex, for example from a storage situation to one associated with structure and cellular metabolism.

The sequential electron micrographs obtained during the embryonic period showed no distinctive changes in structural morphology as incubation proceeded. No comparable studies on the morphology of the avian embryo liver have been performed other than those of Pollak and Shorey (1967), Pollak and Ward (1967) and Schjeide *et al.*, (1973), whose sparse observations were mainly confined to the morphology of the golgi apparatus, endoplasmic reticulum and microsomes. Their findings indicated quantitative changes in the intimate structure of smooth and rough endoplasmic

reticulum as development proceeded, but particularly during the latter half of the incubation period.

In the present work, a standard procedure for the isolation of the major subcellular components via centrifugation has been used following suitable adaptation (Duve de, 1964). However the presence of the extensive cellular cytosolic lipid accumulations, in both the embryo and neonatal chick, required extra caution to be taken to prevent cross contamination of the organelles. This situation clearly only applied to the liver where a large conglomerate of fat was observed at the surface of the supernatant. This layer of fat was carefully removed and analysed, although it did not form part of the supernatant analyses. The major subcellular components normally identifiable with animal tissues were identified throughout the embryonic and post-hatch periods (see Hopkins 1978).

2 Lipid and fatty acid moieties in the subcellular fractions of the liver, heart and brain.

The relative increase in lipid weight shown to be displayed by the mitochondria of the embryo liver during the final week of incubation may be a reflection of a careful regulation, through lipid uptake, to meet the intense lipid metabolic demands of development. Morphological studies however, were not able to delineate any obvious changes in mitochondrial structure. The stabilisation in lipid weights in the liver mitochondria after hatch suggests that organelle maturation had been reached. Fluctuations in lipid weight of the mitochondria within the heart and brain during the embryonic and post-hatch periods also suggest an association with altered cellular activity as a result of metabolic changes during development. The relative changes in lipid weight observed in the microsomes within the tissues following hatching may

similarly suggest cellular adjustments to accommodate the changed metabolic circumstances arising from an independent existence; previous studies on other animal species, for example, have shown that microsomal preparations may differ in composition at various stages of the developmental period (see Berg Van den, 1974).

Observations on other animal systems have also shown that an exchange of phospholipid moieties between subcellular components during embryonic development may give rise to extensive alterations in component concentrations (Wirtz and Zilversmit, 1968; McMurray and Dawson, 1969; Wojczak, Baranska, Zborowski and Drahota, 1971). It must also be borne in mind that the physical manipulations imposed on microsomes during their preparation and isolation may, under certain circumstances, give rise to compositional instability and therefore certain errors.

The major identifiable lipid components of the subcellular fractions studies were in accordance with those expected from previous studies on whole animal tissues (Hsaio and Ungar, 1969; Wood, 1972, 1974; see Noble and Cocchi, 1991). Although differences did exist in the distribution of lipids between the mitochondria and microsomes, the finding of an overall comparatively similar lipid composition suggests that, irrespective of tissue, these organelles have similar functional lipid requirements. The present observations on the embryonic and chick tissues showed a distinct selectivity in the pattern of lipid accumulation by the mitochondria and microsomes; during the embryonic period, phospholipids and free cholesterol had accumulated in preference to cholesteryl esters and triglycerides, whereas following hatching, marked increases in the concentrations of triglycerides in the mitochondria of the liver and heart in paricular, were coincident with a decrease in free cholesterol concentrations. These findings accord with observations on other animal species in which changes in the ratios of free cholesterol: phospholipid were observed during development; an

important role in membrane function and repair was suspected (Baldassare and Silbert, 1979; Rintoul *et al.*, 1979; see Daum, 1985). Where additions to free cholesterol levels have been imposed on liposomal systems through differing lipid substrate exposures, associated changes in cellular membrane permeability have been observed (Oldfield and Chapman, 1971; Kawato *et al.*, 1978). It is possible therefore, that the changes in relative levels of free cholesterol within the mitochondria of the liver, for example, during post-hatch development, may be a response to ensure optimum metabolic function under a rapidly changing biochemical environment.

Despite being relatively insignificant, the slight fluctuation in phospholipid levels observed within the mitochondria and microsomes of all three tissues during the embryonic and post-hatch periods can be speculated upon. It has been shown, in other animal species, that the mitochondria are unable to synthesise certain phospholipids (Wilgram and Kennedy, 1963) and that through an exchange procedure, some phospholipids are derived from the microsomes. Thus, it is possible that the phospholipid changes presently observed are, as in the case of the altered cholesterol levels, a response to adjustments required for developmental and structural purposes, as dictated by the metabolic demands of the embryo.

In contrast to the mitochondria and microsomes, in the supernatant fraction of the liver and heart tissues, cholesteryl esters and triglycerides comprised the bulk of the lipids throughout both the incubation and post-hatch periods. In the brain, the neutral lipids of the supernatant were almost entirely comprised of free cholesterol. High levels of cholesteryl esters within animal cells are unusual, but an exception is that of the steroid hormone producing tissues in which, as in the case of the chick embryo liver, cholesteryl esters are seen to be present as large cytoplasmic droplets (see Myant, 1981; Shand and Noble, 1984). Even in these tissues though, the free

cholesterol ester levels are very much lower than those encountered within the cytoplasm of the chick embryo liver. The presence of substantial proportions of triglycerides within the supernatants of liver, heart and other tissues during the embryonic and post-hatch development periods accords with observations on other animals at similar stages of development (Wheeldon, Shumert and Turner, 1965; Shand and Noble, 1984).

In the liver of the chick embryo, the most important lipid changes are clearly those involving the metabolism and accumulation of cholesteryl esters. As a result of observations on the comparative lipid compositions for the yolk, plasma and tissues of the chick embryo, it has been concluded that the primary function of the cholesteryl esters is associated with the process of yolk lipid uptake through stabilisation of the lipoprotein complex (Vandenheuvel, 1962; Noble and Connor, 1982; Connor, 1993).

From recent studies on the chick embryo, it has been suggested that the lipoprotein interactions and their metabolism are analogous in many aspects to mammalian and human systems (Connor, 1993). Indeed, from mammalian studies, similar mechanisms of intermediary metabolism have already been demonstrated (see Pearce, 1977). From analogy with the evidence which exists on mammalian systems (see Jackson, Morrisett and Gotto 1976; Nilsson-Ehle, Garfinkel and Schotz, 1980; see Mayes, 1981), it has been suggested (Connor, 1993) that the lipoproteins from the yolk sac membrane are synthesised in a nascent (inactive) form and when secreted into the plasma, are deficient in various apoproteins and other structurally important materials. It is commonly accepted from extensive studies that lipid metabolism within the yolk sac membrane involves extensive breakdown and resynthesis of lipoprotein complexes into combinations that are suited for uptake by the embryo (Zacks, 1954; see Schjeide, 1963; Lambson, 1970; see Noble, 1986). Thus, in view

of these findings, it was concluded by Connor (1993) that, once released into the plasma, the nascent lipoproteins quickly associate with other circulatory lipoproteins; rapid transfer of surface material then occurs so that the lipoproteins become structurally complete and are now in their mature form. In proposing such a hypothesis it was suggested (Connor, 1993) that liver high density lipoprotein and apoprotein syntheses were necessary to obtain the correct lipoprotein complex, in particular through the addition of a C2 activator protein or equivalent protein for the activation of lipoprotein lipase and therefore tissue uptake of yolk lipid. The possibility also exists of course that such a protein could be synthesised within the yolk sac membrane and could be transferred to the nascent lipoprotein from other compounds. Alternatively, as has been suggested (Bengtsson, Marklund and Oliverona, 1977), the protein cofactor required for lipoprotein lipase may be derived directly from the yolk.

The presence of extensive lipoprotein lipase activity in certain tissues of the chick embryo at day 14 of incubation, indicates a major role for this enzyme in the tissue specific utilisation of lipoproteins ultimately derived from the yolk material (Speake, Noble and McCartney, 1992). The extensive accumulation of cholesteryl esters within the liver over the last three days of incubation can be explained by the active removal of lipoproteins from the plasma (Yafei and Noble, 1990). The major component of any remnant lipid following tissue lipoprotein lipase activity will clearly be rich in cholesteryl esters since most of the triglycerides in the original lipoproteins will have been depleted by hydrolysis (Shand, West, McCartney, Noble and Speake, 1992; Speake, Noble and McCartney, 1993). Uptake of such remnants by the liver would therefore give rise to the exceptionally high levels of cholesterol ester during the latter part of embryonic development, especially as the embryonic liver also has difficulty in disposing of such material.

The accumulation of such substantial levels of cholesterol ester within the supernatant of the liver makes their exchange with both of the membrane organelles highly probable. Indeed, it was presently observed that as incubation proceeded, the cholesterol ester concentration within the microsomes, in particular, showed a small but significant increase, while such changes, although apparent, were less obvious in the mitochondria. The incorporation of a non polar lipid moiety such as cholesterol ester into the membrane lipids of the mitochondria and microsomes would not be without some effect on their metabolism and membrane fluidity changes have been shown to have a considerable effect on cellular metabolism (Engelhard *et al.*, 1976; see Stubbs and Smith, 1984; Pagano and Longmuir, 1985). With respect to the present studies on the chick embryo liver, it is interesting to note that during the final week of incubation, mitochondrial fatty acid oxidation decreases considerably (Noble *et al.*, 1986b). An association between transfer of fatty acid metabolites across the mitochondrial membrane, their oxidation and changes in membrane fluidity must therefore be considered.

Following hatching, it is clear from the present results that total cholesterol ester levels in the liver rapidly decrease. Previous studies (Noble and Connor, 1984) have shown that just prior to hatching, the proportion of cholesteryl esters within the bile increased dramatically while, at the same time, small but significant increases in the levels of cholesteryl esters were observed to occur within the yolk contents. The probability is therefore, that following hatching, the bile is forced to act as the major pathway for the excretion of the cholesteryl esters accumulated within the liver and for which there is no longer any metabolic function. It is also interesting to note that the rapid removal of cholesteryl esters and exposure to metabolisable dietary lipid components, for example triglycerides, is coincident with an equally rapid

establishment of fatty acid oxidation in the liver (Noble, personal communication). The heart, which does not show any accumulation of cholesteryl esters during the incubation period, shows an increase in triglyceride concentration at the time of hatching. With respect to the present studies, it is interesting to note that, during the final week of incubation, the heart demonstrates a continual and increasing ability to oxidise fatty acid substrates (Noble *et al.*, 1986b).

The upper layer of liver supernatant contained a variable quantity of fat, the level of which clearly depended on the age of the embryo. The greatest amount of this fat was present in livers of the 19 day-old embryos and when analysed, consisted of mainly cholesteryl esters. It is suggested that the fat layer is a conglomerate of several classes of lipoproteins of different densities (Noble and Yafei, 1988). The presence of such substantial proportions of cholesteryl esters is merely a reflection of the final chapter in the specific role of cholesteryl esters for the transport and utilisation of the yolk lipid by the embryo. Its replacement after hatch marks the start of the new phase of neonatal metabolism and growth and the chick's independent existence.

Several notable features associated with the changing fatty acid composition of the subcellular fractions of the tissues can be discerned. There were extensive differences in fatty acid composition between the different lipid moieties within the mitochondria, microsomes and supernatants of the liver and heart. However, apart from minor differences, the specific lipid moieties of the subcellular compartments each showed a similarity in their fatty acid compositions. Specific alterations in fatty acid composition occurred within the subcellular compartments during the incubation and post-hatch periods, these changes being particularly notable within the mitochondria and microsomes. Taken together, the fatty acid compositions of the lipid moieties and changes during incubation and post-hatch were entirely compatible with previous

observations on whole tissues of avian and mammalian species (Noble and Moore, 1967a,b,c; Boland *et al.*, 1974; Wood, 1972, 1974; Noble and Ogunyemi, 1988, 1989). Thus, within the subcellular compartments of all the tissues, phospholipids contained high proportions of unsaturated fatty acids, in particular polyunsaturated fatty acids.

The most notable feature of the three subcellular compartments, but in particular the mitochondria and microsomes, was the distinctive changes which occurred in the proportions of polyunsaturated fatty acids during the incubation and post-hatch periods. At the same time, these changes were commensurate with alterations in the proportions of saturated and monounsaturated species. It is clear that the association between particular combinations of fatty acids changes as incubation and post-hatch proceeds. The proportion and fatty acid composition of any newly synthesised phospholipid within the embryo or chick will be decided by the changing tissue requirement during differentiation.

It is known that in the tissues of the embryonic chick as in all animal species so far studied, the unsaturated fatty acids are esterified predominantly at the sn-2-position (Hawke, 1962; Kuksis and Marai, 1967; Christie and Moore, 1972; see Strickland, 1973). From these studies and in the light of the present observations, it can be concluded that in the liver membranes during embryonic development, there is a partial replacement of palmitic acid by stearic acid at the sn-1-position and a partial replacement of arachidonic acid by linoleic and docosahexaenoic acids at the sn-2-position. The demonstration of curvilinear relationships between the relative concentrations of palmitic versus linoleic acid, palmitic versus arachidonic acid and stearic versus arachidonic acid as development proceeded, inferred a changing

requirement for specific intramolecular fatty acid associations in the subcellular compartments of the liver. Thus, it would appear that in the subcellular organelles of the embryonic chick liver, there is a gradual replacement of an 1-palmitoyl-2-arachidonyl phospholipid structure by 1-steoryl-2-linoleyl and 1-steoryl-2-docosahexonyl phospholipid structures. Although these findings give no indication as to which phospholipid moieties exhibit these changing molecular associations to the greatest extent, from previous studies on embryonic liver (Noble and Moore, 1967a) and the subcellular organelles of ovine placental tissue (Shand and Noble, 1984), it can be suggested that the changes in fatty acids are most likely to occur in the phosphatidycholine fraction. Moreover, there is evidence for the existence of specific intramolecular fatty acid associations in other tissues (Collins, 1963; Moore, Williams and Westgarth, 1965; Deenen Van, Van Golde and Demel, 1966). Relative to the yolk, the phosphatidylcholine fraction of the chick embryo liver increases its content of stearic, arachidonic and docosahexaenoic acids and decreases its concentrations of palmitic and oleic acid as development proceeds (Noble and Moore, 1967a, c). There is therefore, the clear suggestion of alterations within the chick embryo liver in the activities of the two principal pathways involved in the synthesis of phosphatidylcholine (see Strickland, 1973), whereby phosphatidylcholine containing palmitic acid is synthesised by a pathway, which involves 1,2-diglyceride and cytidine diphosphate as intermediates (Kennedy and Weiss, 1956); phosphatidylcholine containing stearic acid is synthesised by methylation of phosphatidylethanolamine (Bremer, Figard and Greenberg, 1960).

The increasing accumulation of stearic and linoleic acids in the liver organelles during the final week of incubation may also be contributed to considerably by a significant change during incubation in the activity of the Δ 6-desaturase system (responsible for the conversion of linoleic to arachidonic acid) within the yolk sac membrane (Noble

and Shand, 1985). The presence of this enzyme in the yolk sac membrane plays a major role in the polyunsaturated fatty acid pattern of the developing embryo's tissues. Thus, the metabolic regression of the yolk sac membrane as hatching approaches (Noble and Shand, 1985), would inevitably lead to a reduction in embryonic access to arachidonic acid. There is a concomitant increase in the relative proportions of linoleic acid. Likewise, the established presence in the yolk sac membrane of an active Δ9-desaturase system (responsible for the conversion of stearic to oleic acid) would make a considerable contribution to the oleic acid requirements of the embryo. A demise in Δ 9-desaturation activity, with the approach of hatching would, in a similar manner, give rise to an increased embryonic access to stearic acid at the expense of oleic acid.

In contrast to the liver, during the embryonic period, there were no obvious changes in fatty acid compositions in the phospholipid fractions of the heart organelles. Compared with the liver, the heart organelles were characterised by higher levels of oleic acid and lower levels of linoleic acid throughout the incubation period. Following hatching however, the fatty acid compositions of the subcellular compartments of both tissues showed extensive similarities. Previous studies on avians have shown there to be both close similarities and certain distinctive differences between the tissues of liver and heart (Wood, 1972, 1974; see Noble and Cocchi, 1990), although limited data are available on the lipid compositions of these organelles (Ward and Pollak, 1967; Nakagawa *et al.*, 1982). During the early post-hatch period the organelles of both displayed a rapid increase and decrease respectively in their levels of C18 and C20 fatty acids; it can be concluded that such changes were in response to the elimination of the yolk sac remnant (Noble and Ogunyemi, 1988, 1989).

An increase in the concentration of docosahexaenoic acid in the liver organelles at the time of hatching is interesting and indeed, such a finding accords with that reported previously on whole liver tissue (Ogunyemi, 1987; Noble and Ogunyemi, 1989). The high concentration of this fatty acid during embryonic development can be accounted for by the preferential absorption of phosphatidylethanolamine species rich in docosahexaenoic acid (Noble and Moore, 1967b,c) and possibly, although not yet proven, of increased Δ5- and Δ4-desaturase activities within the yolk sac membrane. The question remains as to the source of docosahexaenoic acid following hatching.

The changing spectra of fatty acids during the incubation and early post-hatch periods, in particular, those involving the longer chain polyunsaturated fatty acids, would affect, to some extent, membrane fluidity (see Spector and Yorek, 1985; see Stubbs and Smith, 1984; Quinn *et al.*, 1989). This same membrane fluidity has, in turn, been shown to affect metabolism (Sinensky *et al.*, 1979; see Brenner, 1984; Pagano and Longmuir, 1985; see Daum, 1985; see Spector and Yorek, 1985) and as can be suggested, may account for the considerable decrease in liver mitochondrial oxidation observed during the final weeks of incubation (Noble *et al.*, 1986b).

Phospholipids contributed by far the major proportion of the lipids and contained a very high concentration of both C20 and C22 fatty acids; these polyunsaturated fatty acids have been shown to dominate the phosphatidylethanolamine fraction and play an essential role in nervous tissue development (Bernsohn and Cohen, 1972; Bourre, Faivre, Dumont, Nouvelot, Loudes, Puymirat and Tixier-Vidal, 1983). In contrast to the liver and heart, where there was a predominance of arachidonic acid during the final week of incubation, the organelles of the brain demonstrated extremely high levels of docosahexaenoic acid, a feature maintained through the post-hatch period as

well. Previous studies on avian brain tissue have shown that there is an increase in the level of docosahexaenoic acid, particularly at around the twelfth day of incubation (Miyamoto *et al.*, 1967; Gonzalez-Ros and Ribera, 1980; Bordoni *et al.*, 1986) and coinciding with a peak of polyneural discharge; studies on mammalian systems have demonstrated a similar observation between docosahexaenoic acid and brain development (see Turchetto and Pignatti, 1982).

In the present observations, a decrease in the relative concentration of docosahexaenoic acid in the brain microsomes during the early post-hatch period suggests an increase in turnover rate of this acid. Studies on mammalian species have shown a decrease in Δ6-desaturase activity in the brain as developmental age increased (Bordoni *et al.*, 1988; Bourre, Piciotti and Dumont, 1990), but in contrast to these findings, Anderson *et al.* (1989, 1990) have suggested an enhanced activity of the desaturase enzymes within the brain, retina and other neural tissues of the chick following hatching. It must be noted however, that the present drop in docosahexaenoic acid levels in the brain microsomes represents the total phospholipid fractions; the effect may be somewhat smaller compared with that which would have been observed in individual classes of phospholipid. In contrast to the liver and heart, the relative concentrations of the linoleic and linolenic acids were low in the brain organelles, thereby according with previous observations on both mammalian and avian species (Tahin *et al.*, 1981; Bourre, Pascal, Durand, Masson, Dumont and Piciotti; 1984; Bordoni *et al.*, 1986; Youyou, Durand, Pascal, Piciotti, Dumont and Bourre, 1986).

The phospholipid classes within the embryonic tissue exhibited a characteristic fatty acid composition differing entirely from that of the yolk (Noble and Moore, 1967a,b,c). The differences were such that only a small percentage of the phospholipids of the yolk would meet the embryonic molecular specifications without hydrolysis and reesterification. The results of comparative studies on the fatty acid composition of individual phospholipids within the yolk, yolk sac membrane, liver and extrahepatic tissues (Noble and Moore, 1965b, 1967a, c; Wood, 1974; Abad *et al.*, 1976; Nakagawa *et al.*, 1982) are consistent with extensive degradation of the yolk phospholipids during assimilation, followed by their resynthesis into products suited for development; some oxidation to furnish energy is also possible (Budowski *et al.*, 1961). The presence of Δ6-and Δ9-desaturase activities within the yolk sac membrane (Noble and Shand, 1985) was able to be correlated with the levels of monounsaturated and polyunsaturated fatty acids in the yolk, yolk sac membrane and liver during embryonic development (Noble and Moore, 1967a, b,c; see Noble, 1986, 1987b; see Noble and Cocchi, 1991).

With respect to the brain organelles, the presence of small amounts of 11, 14 eicosenoic (1-1.5 per cent) and docosatetraenoic acids (1-1.5 per cent) is interesting; these polyunsaturated fatty acids were not detected in the yolk sac membrane and subcellular organelles of the liver. The ability of the developing brain for Δ6-, Δ5- and Δ4-desaturase activity is evident from studies *in vivo* (Sinclair, 1975; Dhopeshwarkar and Subramanian, 1975, 1976; Cook, 1980) and *in vitro* on mammalian species (Strouve-Vallet and Pascaud, 1971; Cook, 1978). Further studies have shown that the retina itself is able to synthesise its own docosahexaenoic acid *in vivo* from the n-3 fatty acids which must be available (Bázan, Careaga, Sprecher and Bázan, 1982). Thus, according to the commonly accepted pathway, the metabolism

of linoleate and linolenate both require the sequential use of Δ 6-, Δ 5- and Δ 4-desaturases. However, from the general experimental approaches that have been used to show that the microsomes contain three specific desaturases, all the available evidence is consistent with the presence of separate Δ 5- and Δ 6-desaturases; there is no direct evidence supporting the commonly accepted hypothesis for an acyl CoA-dependent Δ 4- desaturase (Strouve-Vallet and Pascaud, 1971; Dhopeshwarkar and Subramanian, 1975, 1976; Cook, 1978). From recent studies on rat hepatocytes, it has been confirmed that the direct precursor (docosapentaenoic acid) for docosahexaenoic acid was indeed metabolised to the expected product but the results showed that the pathway was independent of a Δ 4-desaturation step (Voss, Renhardt, Sankarappa and Sprecher, 1991). Furthermore, this particular study suggested that the pathway of fatty acid synthesis involved the microsomal chain elongation of docosapentaenoic acid (n-3) to tetradocosapentaenoic acid (24:5 (n-3)), followed by its desaturation to tetradocosahexaenoic acid (24:6 (n-3)). This microsomal product was then metabolised, via ß oxidation to docosahexaenoic acid (n-3). The existence of such a mechanism must therefore be considered in the brain for the formation of docosatetraenoic and docosahexaenoic acids, since the ability of the brain microsomes for the two carbon chain elongation steps is evident from many studies (Bernsohn and Cohen, 1972; Fewster, Ihrig and Mead, 1975; Sinclair, 1975; Dhopeshwarkar and Subramanian, 1975, 1976; Cook, 1982).

The observation that Δ 6- and Δ 9-desaturase activities do not peak during myelination is surprising (Carreau, Daudu, Mazliak and Bourre, 1979; Bourre *et al.*, 1990). In contrast to the desaturase activities, it has been shown that in mammalian brains, the levels of saturated and mono- and polyunsaturated fatty acids peak during myelination (Bourre, Paturneau-Jouas, Daudu and Baumann, 1977). Such a finding must

obviously pose the question as to when these polyunsaturated fatty acids are synthesised. There is strong evidence that the developing brain has a high capacity for desaturation (Cook, 1978, 1982). Despite this capacity however, it is not clear as to what extent docosahexaenoic acid is derived specifically from the brain metabolism of linolenic acid. It would appear from mammalian studies, that the placenta plays a considerable role in the provision of the essential polyunsaturated fatty acids to the developing foetus; an extensive ability for the placenta to synthesise C20 and C22 fatty acids has been demonstrated (Wahle, 1974; Noble *et al.*, 1985).

The form in which the dietary docosahexaenoic acid reaches the brain is unknown but from mammalian studies, the possibility exists that docosahexaenoic acid may be derived directly from the diet through the action of brain lipoprotein lipase on triglyceride-rich lipoproteins (Chajek, Stein and Stein, 1977). However, recent studies on avian tissues, have confirmed that lipoprotein lipase activity was low in the brain of the developing embryo (Speake *et al.*, 1992). Furthermore, lipoprotein lipase was inhibited by triglycerides rich in polyunsaturated fatty acids (Speake *et al.*, 1993). Since most of the circulating docosahexaenoic acid in the brain of the chick embryo is present in the phospholipids and not triglycerides (Bordoni *et al.*, 1986; Noble and Cocchi, 1989a), the possibility exists that there is a direct transfer of intact phospholipids into the brain. It is suggested therefore that the polyunsaturated fatty acids required for myelination accumulate in oligodendrocytes before myelination or are supplied from the bloodstream.

Studies on mammalian cells have shown that the permeability properties of subcellular membranes can be markedly influenced by their fatty acid composition and in particular, by their degree of unsaturation (Gier de *et al.*, 1968; see Brenner, 1984;

Berlin *et al.*, 1989; see Quinn *et al.*, 1989). Present observations showing extensive changes in fatty acid chain length and degree of unsaturation in the liver organelles during the embryonic and post-hatch period may suggest that the changes in the mitochondrial and microsomal membranes are necessary to maintain the correct fluidity of the liquid phase. Modifications to membrane fatty acid composition can be extensive enough to promote an alteration in a range of functions which include carrier mediated transport (Burns *et al.*, 1979; Yorek *et al.*, 1984), the properties of certain membrane bound enzymes (Engelhard *et al.*, 1976; Sinensky *et al.*, 1979; Pagano and Longmiur, 1985), cell growth (Doi *et al.*, 1978; Spector *et al.*, 1979) and a range of other functions as detailed in the Introduction. It is therefore possible that alterations in fatty acid composition, unless compensated for, would not be without some effect on the cell metabolism.

The extent of substitution of palmitic acid by stearic acid in the liver membranes during the final week of incubation results in a significant increase in the saturated chain length; such long chain saturated fatty acids have the ability to form tightly packed bilayers with relatively low permeability (Chen *et al.*, 1971). Previous studies have also shown however, that with the introduction of more highly unsaturated fatty acids, expected proportional increases in membrane fluidity did not occur (Coolbear *et al.*, 1983). Thus, although arachidonic acid has four double bonds, its introduction into the membrane leads to an increase in transition temperature since it becomes difficult to pack polyunsaturated chains into the closely packed 'gel' phase. Similarly docosahexaenoic acid, by virtue of its high degree of unsaturation, has been shown to evoke an unexpected decrease in membrane permeability (see Brenner, 1984).

In the subcellular membranes of the heart, the changes in docosahexaenoic acid concentrations were small compared with other fatty acids and agreed with previous studies on avians (Wood, 1974). It is interesting to note that in contrast to the liver, the membranes of the heart mitochondria, undergo a decrease in the relative concentration of docosahexaenoic acid at the time of hatching. Furthermore, previous studies have also shown that, in contrast to the liver, there is an increase in heart mitochondrial oxidation during the final week of incubation (Noble *et al.*, 1986b).

In addition to the length of the fatty acyl chain and the degree of unsaturation, membrane permeability depends also on the class of phospholipid. Phosphatidycholine has a lower transition temperature than phosphatidyethanolamine (see Lee, 1983; see Brenner, 1984). Differences in metabolic function may account for the preferential accumulation of phosphatidylcholine in the microsomes of many mammalian tissues, whilst the mitochondria favour a relatively high concentration of phosphatidylethanolamine (Bartley, Getz, Notton and Renshaw, 1962; Getz *et al.*, 1964; Colbeau *et al.*, 1971). This feature may also go some way to explain the occurrence of significant changes in phospholipid species with developmental status, such as previously shown to occur in avian heart tissue (Wood, 1974). In tumour cells however, several studies have shown that there were no apparent changes in membrane fluidity in spite of extensive polar head group modification (Schroeder, Perlmutter, Glaser and Vagelos, 1976; Esko, Gilmore and Glaser, 1977; Schroeder, 1978), including associated changes in fatty acid chain length and degree of unsaturation (Schroeder, 1978).

In the context of the present observations, it may be suggested that changes in the phospholipid species, together with the obvious alterations in fatty acid chain length and unsaturation, are directed towards the maintenance of relative consistency in the physical properties of the liquid phase of the membranes during embryonic and post-hatch development. However, in spite of the changes being able to provide a compensatory mechanism enabling the organelles of the tissues to maintain their membrane fluidity within narrow limits, a process known as 'homoviscous adaptation' (Sinensky, 1974), such compensations may be insufficient to prevent adverse physical changes occurring in other membrane components. Thus, in the present circumstances, an increase in the level of docosahexaenoic acid in the liver mitochondria at the time of hatching may not be adequately compensated for by appropriate changes in the levels of oleic and linoleic fatty acids, with consequential effects on vital aspects of metabolism, for example, fatty acid oxidation (Noble *et al.*, 1986b).

Although the triglycerides represent a diminishing proportion of the total lipid in the liver during embryonic development, a marked feature of the liver triglycerides in particular and of the plasma and several other major extrahepatic tissues, is the very high concentration of docosahexaenoic acid (Noble and Moore, 1967b; see Noble, 1986). This feature is not exhibited by the yolk contents and is unique, since it is not found in the triglycerides of other terrestrial animals (Hilditch and Paul, 1938; see Noble, 1980, 1981b). Indeed, the level of docosahexaenoic acid in the liver triglycerides during embryonic development is greater than in the phospholipids (Noble and Moore, 1964; see Noble, 1986).

Apart from synthesis within the yolk sac membrane from C18 precursors (Noble and Shand, 1985), the accumulation of docosahexaenoic acid in the embryonic tissues is also the result of a preferential absorption by the yolk sac membrane of phosphatidylethanolamine species that are rich in this acid (Noble and Moore, 1967b,c). The transfer of fatty acids from the triglycerides of plasma lipoproteins is mediated by lipoprotein lipase which is present at the luminal surface of the blood capillaries of specific tissues and catalyses the hydrolysis of the triglycerides present in the plasma chylomicrons and very low density lipoproteins (Speake, Parkin and Robinson, 1985).

Most recently Speake *et al* (1993) have proposed that the fatty acid composition of the triglycerides associated with specific tissues within the chick embryo is affected by the substrate specificity of the lipoprotein lipase. Previous studies have shown that esters of arachidonic (Nilsson, Landin and Schotz, 1987; Ridgway and Dolphin 1984; Melin, Qi, Bengtsson-Olivercrona, Akesson and Nilsson 1991), eicosapentaenoic (Melin *et al.*, 1991), and docosahexaenoic (Melin *et al.*, 1991) acids in the triglycerides are relatively resistant to hydrolysis by lipoprotein lipase.

Results obtained for the avian embryo are compatible with such findings and have been able to explain the high accumulation of docosahexaenoic acid within specific tissues, including for example, the liver. The level of docosahexaenoic acid in the triglycerides of tissues which expressed lipoprotein lipase activity, was considerably less than that present in the plasma triglycerides of the chick embryo (Speake *et al.*, 1992, 1993; Noble and Speake, personal communication). It was therefore suggested that the high proportion of docosahexaenoic acid in the liver triglycerides, especially of the chick embryo, arose as a consequence of the hepatic uptake of remnant particles

produced by the action of lipoprotein lipase on the triglyceride-rich plasma lipoproteins (Speake *et al.*, 1993). In addition, certain key tissues of the chick embryo failed to show lipoprotein lipase activity, in particular the liver (Speake *et al.*, 1993) and consequently, any absence of this enzyme would lead to a further enhancement in the docosahexaenoic acid levels of the triglycerides present.

From the present investigations on the triglyceride fraction of the liver, it is clear that there is a rapid decline in the concentrations of both arachidonic and docosahexaenoic acids within forty-eight hours of post-hatching. The reliance, after hatching, on a food source with a high lipid content and the ability of a broiler chicken to gain weight, necessitates that the emergent chick quickly possesses the ability to rapidly absorb and metabolise available dietary lipid over a short period of time. Although the concentration of lipid per unit weight of dry matter in the liver decreased following hatching, the relative concentrations within the total lipid of the triglycerides increased and were accompanied by rapid changes in fatty acid composition from a highly polyunsaturated situation to one containing a far more saturated species (Noble and Ogunyemi, 1989).

Lipid metabolism in the newly-hatched chick has to undergo a rapid change to accommodate, firstly, its independent existence and secondly, the large amounts of lipid being provided from the diet and the diminishing amounts being provided by the remnant yolk sac material (Noble and Ogunyemi, 1989). Changes in lipid and fatty acid composition, together with the rapid establishment of an extensive lipogenic capacity (Goodridge, 1968, 1970; Goodridge, Garay and Silpananta, 1974; Ryder and Campos, 1979), indicate the extent of alteration to the lipid metabolism of the liver at a time when the need for tissue growth and synthesis is being rapidly

increased. An increasing role of the desaturases (Noble and Shand, 1985) and the disparity between the polyunsaturated fatty acids of the remnant yolk sac material and those of the diet, are also of importance.

In addition, with respect to the longer chain polyunsaturated fatty acids, it is highly probable following hatching, that tissues other than the liver, are also acquiring an extensive capacity of their own for the synthesis of lipid for both structural and storage purposes which will quickly outweigh the substantial but diminishing contribution of lipid still being made by the residual yolk. The initial high levels of docosahexaenoic acid in the triglycerides of the embryo and at hatching would provide a source of those essential components for the triglyceride and phospholipid requirements of other tissues. Appropriately, such changes would lead to a reduction in docosahexaenoic acid levels within the triglycerides of the liver, with consequential effects on subcellular membrane fatty acid composition and metabolism both in the liver and other tissues.

A distinguishing feature of the cholesteryl esters of the liver fractions was their very high content of oleic acid, greater than 60 per cent of the total lipid present. This finding correlates with the very high levels of cholesterol oleate observed to accumulate in the yolk, yolk sac membrane and liver (Noble and Moore, 1964, 1966, 1967b) during development. By comparison with the subcellular fractions of the liver, those of the heart and brain displayed significantly lower levels of oleic acid. The relative concentrations of oleic acid increased in the cholesteryl esters of the liver subcellular membranes at the initial stages of yolk lipid uptake, that is, between days 13 and 16 of incubation; in common with the heart and brain there was also an increase in oleic acid during early post-hatch.

Recent studies have shown that cholesterol oleate present within the liver during embryonic development is synthesised in the yolk sac membrane (Noble and Connor, 1982; Noble *et al.*, 1984). Cholesterol oleate plays an important role in the assembly of the lipoprotein complex required for the transfer of lipid from the yolk to the embryonic tissues and for tissue function itself (Noble and Moore, 1967b; Noble *et al.*, 1984; see Noble, 1986, 1987b; Yafei and Noble, 1990). The cholesterol ester forms a 'wedge shaped' planar molecule that can be found in the inner core of lipoproteins situated between the phospholipid fatty acid tails where its function is to promote order and stability of the complex (Vandenheuval, 1962). As previous studies have shown, cholesterol oleate promotes order in the concave phospholipid bilayer by occupying gaps between the phospholipid fatty acid tails and thus, inhibiting molecular rotation and molecular bumping (Vandenheuval, 1962). The overall effect of cholesterol oleate is to stabilise the radius of curvature and diameter of the lipoprotein complex. Deposition of oleic acid in the membrane has a consequential effect on the membrane transition temperature due to its genetic configuration (Coolbeau *et al.*, 1983). As cholesterol ester is a non polar molecule, the possibility exists that the binding effect of oleic acid in the membrane is considerably enhanced.

The very high level of cholesterol ester displayed by the embryonic liver was mainly associated with the supernatant; its level following hatching was rapidly reduced and replaced by triglycerides. Following hatching the only unique feature to remain that was associated with the embryonic period was a relatively high level of oleic acid within the cholesteryl esters; this was in spite of the overall dramatic decrease in cholesterol ester level. The continuing high levels of oleic acid in the cholesteryl esters of the membranes observed during post-hatch development may be associated with the continuing, but diminishing absorption of the yolk complex (Noble and

Moore, 1967b; Noble and Connor, 1982; Noble *et al.*, 1984), or a significantly lower metabolic turnover of cholesterol ester relative to the other major lipid fractions (Moore, Noble and Steel, 1969; Cook, Scott, Faichney and Davies, 1972; see Noble, 1981b).

Recently, more specific details have been obtained on the cholesterol esterification processes leading to cholesterol ester accumulation in the liver during both embryonic and post-hatch periods (Shand *et al.*, 1992). The presence of substantial acylCoA: cholesterol acyltransferase activity in the yolk sac membrane during embryonic development was much higher compared with other tissues. In contrast, the activity of microsomal cholesterol ester hydrolase in the yolk sac membrane was low during this period, but rose slightly just before hatching. High levels of cholesterol ester hydrolase inhibitor protein were also observed throughout embryonic development, sufficient to prevent cholesterol ester hydrolysis in the cytosol. At hatching, acylCoA: cholesterol acyltransferase activity decreased, whilst the activity of the microsomal cholesterol ester hydrolase increased and low levels of inhibitor protein allowed cholesterol ester synthesis to be switched off. These studies confirmed therefore, that during embryonic development, the relevant enzymic systems in the yolk sac membrane are very strongly directed towards the esterification of free cholesterol. After hatch cholesterol esterification is no longer required and the enzymes necessary for cholesterol ester hydrolysis are activated (Shand *et al.*, 1992).

In summary, extensive investigations on the last seven days of incubation, have shown that the uptake of yolk lipid is characterised by distinctive changes in lipid and fatty acid compositions; associated alterations to subcellular compositions occurred with related effects on metabolic activity. Yolk lipid performs a role both as an energy

source and as a supply of nutritionally essential tissue components, and its transfer into the embryo constitutes an important part of the interrelated chain of events that ends with the successful emergence of the chick.

Immediately following hatching, the tissues of the chick acquire an extensive capacity of their own for the synthesis of lipid for both structural and storage purposes that quickly outweigh the substantial but diminishing contribution to lipid accumulation still being made by the yolk. The newly-hatched chick undergoes a rapid change in metabolism imposed on it in order to cope with its independent existence. The changes in the lipid and fatty acid compositions, both at the cellular and subcellular levels observed to occur in the liver of the newly-hatched chick, and to a lesser extent, the heart and brain, indicate a rapidly changing regime of lipid metabolism and an increasing contribution of lipid being provided by the diet.

The present investigations at the subcellular level have revealed changes in the lipid and fatty acid compositions. Such changes are undoubtedly associated with cellular physiology and metabolic regulation and the utilisation of lipid metabolites during yolk absorption. There are indications that membrane fluidity in particular, may be altered through the rapidly changing lipid and fatty acid environment, leading to associated effects on metabolism.

3 Parental age and lipid metabolism.

The last forty years have seen a period of rapid growth and expansion in the broiler industry. In 1981, domestic poultry meat accounted for 22 per cent of the total domestic meat consumed, but between 1981 and 1989, the consumption of domestic poultry meat increased by 42 per cent (from its 1981 level) and peaked in 1988, at 25

per cent of total meat consumed (see FAWC report, 1992). At present, the increase in consumption of poultry meat as a proportion of the total meat eaten is 6 per cent per annum, this rise being achieved at the expense of red meat and pork. Significant developments during this time have included the genetic improvement of growing stock, controlled environment housing and the increased sale of portioned and further processed chicken products.

One of the main reasons for these recent changes in meat consumption is based on evidence which links high levels of saturated fat in the diet to coronary heart disease (Phillips, Lemon, Beeson and Kuzma, 1978; Mattson and Grundy, 1985; Miller, Martin, Webster, Wilkes, Miller, Wilkinson and Meade, 1986). In the case of the chicken, the public has the belief that meat off the carcass is low in fat and that any fat which is present, is of good quality. Scientific reports linking diet and health (see reports from NACNE, 1983; COMA, 1984; MAFF, 1988) have made a considerable impact on the public; the belief by the consumer that a decrease in fat consumption provides the key to better health has increased the demand for low fat products.

Recently, extensive coverage by the food industry, British Medical Association, media and consumer activists, has led to a greater, although somewhat confused, awareness by the public of the importance of mono- and polyunsaturated fatty acids in the diet and their respective roles in the prevention of heart disease (Kromhout, Bossscheiter and Coulander, 1985). The additional confirmation that poultry meat is relatively low in fat (see Holland, Welch, Unwin, Buss, Paul and Southgate, 1991) and a better source of monounsaturated and polyunsaturated fatty acids, compared with other domestic meats (Noble, personal communication), has led to its increasing acceptance by the public, as a 'healthy' food. In the broiler industry therefore, commercial

pressures have dictated that fertile eggs should be taken from the parent broiler breeder from as early an age as possible. Where this has been practised, poorer hatchability (60 per cent compared with an acceptable level of 90 per cent) and increased levels of chick mortality (10 per cent compared with a level of 2 per cent under normal commercial practice) are posing considerable problems for the industry (McNaughton *et al.*, 1978; Shanawany, 1984).

The age of the parent bird has a significant effect on embryo hatchability and chick survival. Previous studies on the normal embryo have drawn attention to the dramatic increase in lipid utilisation and respiration associated with rapid embryonic growth over the last week of incubation (Noble and Moore, 1967a,b,c; Noble *et al.*, 1984; see Noble, 1986; see Noble and Cocchi, 1991). More recently, it has become clear that the effect of parental age on hatchability and chick survival is associated with changes in both these areas of metabolism (Shanawany, 1984; Noble *et al.*, 1986a, 1986b; Noble and Yafei, 1988; Yafei and Noble, 1990; Noble and Speake, personal communication). Investigations have shown that the reduced hatchability displayed by the eggs from these very young broiler-breeders (that is twenty-three to twenty-eight weeks of age) was associated with an abnormal distribution of overall egg weight between the embryo and its associated yolk tissues during incubation (Shanawany, 1984).

Biochemical and morphological studies undertaken more recently have shown that this abnormal distribution of egg weight between the embryo and its yolk tissues,stems from a combination of the less efficient deposition of essential lipid material at point of lay, with consequential disturbances to tissue lipid and fatty acid compositions and subsequent lipid metabolic effects (Noble *et al.*, 1986a, 1986b; Noble and Yafei, 1988; Tullett and Noble, 1988a; Yafei and Noble, 1990; Noble and Speake, personal

communication). Alterations in metabolic activity, for example, were associated with changes in hepatic lipid oxidation (Noble *et al.*, 1986b) and carbon dioxide evolution and subsequent accumulation in the air space (Tullett and Noble, 1988a), both of which led to consequential effects upon pipping and chick emergence. In addition, the respiration of the embryo may be impaired by both shell conductance and the incubator environment (Tullett and Noble, 1988b).

The morphological studies undertaken presently were not able to delineate any obvious changes in the mitochondrial structure between offspring from mature and young parents. Thus the mitochondria, from an early age, took on the appearance of being structurally mature. However obvious differences in lipid and fatty acid compositions of various selected tissues, between offspring from mature and young parents, were able to be confirmed. Compared with offspring from mature parents, the weight of total lipid in the mitochondria and microsomes of the liver and to a lesser extent, the heart and brain, was higher in offspring from young parents during the embryonic and post-hatch periods. Such an observation, at first sight, would seem contradictory to that expected in view of recent findings which have shown a reduced lipid transfer at the cellular and subcellular levels in tissues of offspring from young parents (Noble *et al.*, 1986a; Yafei and Noble, 1990). Nevertheless, earlier observations on subcellular membranes have suggested that the apparent absence of matrix material in the young mitochondrion may account for mitochondrial membranes of an unusually high lipid content (Goldho, 1968); once the post-hatch stage is reached, the mitochondrial membranes themselves may be relatively rich in protein.

It is suggested therefore, that the presence of a much higher concentration of lipid, particularly in the liver mitochondria during the embryonic and post-hatch periods, is indicative of a more immature membrane. Such a finding accords with the suggestion that a low protein: lipid ratio is a feature typical of more primitive cells (Schjeide *et al.*, 1970). The possibility that the mitochondria may have a large amount of matrix but relatively poor cristae development at the post-hatching stage as compared with the mitochondria at the adult stage, is speculative (Goldho, 1968); the present results would suggest that the cristae were generally well developed in both groups of offspring at the time of hatching.

In the present investigations, discernible differences in subcellular tissue lipid compositions between embryos and chicks from mature and young parents were slight. Nevertheless, the present results for the embryo and chick subcellular fractions are compatible with previous observations on whole tissues (Noble *et al.*, 1986a; Noble and Yafei, 1988; Yafei and Noble, 1990). Thus, the lower concentration of free cholesterol present in the liver mitochondria of embryos and chicks from young parents could arise as a consequence of a lower level of free cholesterol present in the yolk lipid at point of lay (Noble *et al.*, 1986a). The differences in lipid composition of the heart tissue also accorded with previous observations (Noble *et al.*, 1986a).

Alterations in the cholesterol ester: triglyceride ratio in the yolk sac membrane of embryos from young parents have already been confirmed (Noble *et al.*, 1986a). Such changes would undoubtedly lead to a difference in the species of lipoprotein being transferred from the yolk sac membrane and subsequent alterations in rate of transport to the embryonic tissues. A reduction in lipoprotein levels has been observed within the plasma of embryos from young parents (Yafei and Noble, 1990).

Results obtained recently on the cholesterol esterification processes (Shand *et al.*, 1992; Noble and Shand, personal communication) tend to suggest that a delay in the yolk lipid uptake may arise as a result of inherent changes in the activity of several major enzymes in the yolk sac membrane (Noble, personal communication). Evidence has been presented to show that in the embryos from young parents, the specific activity of a cholesterol ester hydrolase inhibitor protein is significantly lower throughout development than in embryos from mature parents (Noble and Shand, personal communication). The difference would be sufficient to inhibit the attainment of necessary cholesterol ester levels in the cytosol of the yolk sac membrane for yolk lipid uptake. Clearly, the changes in lipid uptake are such as to exert a considerable effect on both the utilisation of primary lipid metabolites during yolk absorption and associated effects on lipid metabolism. With regards to the two embryo groups, the existence of such a situation through incubation suggests that, although chronologically the two embryos are the same, metabolically they are not.

The recent observations of Speake *et al.* (1993) have shown that lipoprotein lipase activity discriminates between triglycerides that are rich in polyunsaturated fatty acids during embryo development and those which are highly saturated. The triglycerides associated with the yolk sac membrane are more unsaturated during early embryonic development (Noble, personal communication). Thus, in relative terms, there is a higher discrimination between lipid uptake by the adipose tissue with more lipid being diverted towards the liver during early incubation (Noble, personal communication). Lipid uptake increases in the adipose tissue as the fatty acid component of the triglycerides becomes more saturated.

The present studies on the subcellular tissue fractions highlight obvious differences in fatty acid compositions between embryos and chicks from the two parental ages. The liver in particular showed these changes. Within the liver organelles, the major changes in fatty acid composition were displayed by the phospholipids of the mitochondra and were associated in particular, with lower levels of palmitic and stearic acids and higher levels of arachidonic and docosahexaenoic acids in embryos from young parents. As has already been pointed out, changes in normal embryonic lipid development are associated with alterations to specific fatty acid combinations within the phospholipids (see Section 7.1 of the Results). Thus, it can be suggested that in the embryos from young parents, such changes are slower to occur. Failure to bring about these changes in fatty acid combinations, together with differences in free cholesterol: phospholipid ratio (see Section 5.1 of the Results) would imply differences in membrane fluidity with consequential effects on cell metabolism. It is interesting to note, that in embryos from young parents, in contrast to those from mature parents, the liver demonstrates a continual ability to oxidise fatty acid substrates during the final week of incubation (Noble *et al.*, 1986b).

The present observations on embryos from young parents suggest some malfunction in the process of lipid assimilation by the yolk sac membrane. Observations in related areas would support such a conclusion. Studies have confirmed that the yolk sac membrane is the principal source of cholesteryl esters necessary for lipid transport and successful embryonic development (Noble and Connor, 1982; Noble *et al.*, 1984). The reason for the preferential association of cholesterol, in particular with oleic acid in the yolk sac membrane, remains unknown. However, evidence is emerging (Shand *et al.*, 1992) that the cholesterol ester plays a necessary functional role in the assembly of the lipoprotein complexes required for the successful transfer of lipid from the yolk

and assimilation by the embryo. In view of this finding (Shand *et al.*, 1992), it has been suggested that any changes to the esterification process could affect the overall lipid transport and subsequent embryonic metabolism (Noble, personal communication). Evidence of this association exists from investigations in which malfunctions in the process of yolk lipid uptake were associated with alteration in the proportions of cholesterol ester, relative to the other major lipid fractions in both extra and intra-embryonic tissues (Noble *et al.* 1986a; Yafei and Noble, 1990). Earlier observations have also suggested a specific role of cholesteryl esters in the adequate uptake of yolk lipid and embryo survival (Schjeide, 1963).

As noted previously (see p141), cholesterol ester levels in both the yolk sac membrane and embryonic tissues are regulated by the balance between cholesterol esterification and cholesterol ester hydrolysis brought about respectively by the action of acylCoA: cholesterol acyltransferase and cholesterol ester hydrolase (Noble and Connor, 1982; Noble *et al.*, 1984; see Suckling and Stange, 1985; West and Shand, 1991; Shand *et al.*, 1992). Most recently, the level of cholesterol ester hydrolase has been shown to be dependent upon the levels of an inhibitor protein present within the yolk sac membrane (Shand *et al.*, 1992). Although the origin of this protein is as yet to be proved, it may be speculated from levels during incubation, that it is synthesised in the yolk sac membrane (Noble, personal communication). Evidence of an association between levels of this inhibitor protein within the yolk sac membrane and parental age have recently come to fore (Noble and Speake, personal communication). The low levels of inhibitor protein in the yolk sac membrane, identified in embryos from young parents (Noble and Speake, personal communication), would suggest an inability to suppress cholesterol ester hydrolase activity and therefore, a consequential reduction in cholesterol ester accumulation and a reduction in lipid assimilation.

A feature of the present observations on organelles lipid compositions within the liver mitochondria, was a low level of free cholesterol. From previous work, it has been shown that fertile eggs from young parent stock exhibit lower levels of free cholesterol in the eggs when laid (Noble *et al.*, 1986a). Thus, this feature, in combination with a lowered ability to reach cholesterol ester levels within the yolk sac membrane, may well be of significant importance in survivability.

It has also been shown that of all the embryonic tissues, only the brain relies solely on *de novo* synthesis, rather than the free cholesterol directly derived from the yolk (Connor *et al.*, 1969; Jain *et al.*, 1969; Svanberg, 1970; Wong and Lennarz, 1982). In the circumstances of the low levels of yolk free cholesterol, already outlined, the brain is the only tissue which may have the capabilities to compensate for the diminished yolk lipid transfer. Indeed, present observations on brain lipid organelle compositions have shown a higher level of free cholesterol, in absolute terms, in the mitochondria and microsomes of progeny from young parents stock. In other tissues, it can be proposed that the inability to derive sufficient free cholesterol from the yolk, will lead to inadequacies in membrane structure with detrimental effects on a wide range of metabolic features. Faced with such a problem, the tissue may attempt to compensate by utilising alternative sterols which are chemically adequate, but physiologically inadequate. Indeed, under conditions where cholesterol levels in the yolk have been reduced by the action of certain hypocholesterolemic drugs, changes in both hatchability and sterol ester composition have been observed (see Naber, 1976).

As stated previously (p122), maturation of the lipoprotein complexes within the yolk sac membrane and their subsequent utilisation by the embryo, depends upon the correct levels of associated apoproteins (see Pearce, 1977; Connor, 1993). In the

absence of apoprotein C2, the major lipid carrying lipoprotein complexes, that is, those of very low density, are unable to be further metabolised by the lipoprotein lipase enzymes within the tissues (Connor, 1993). Current investigations (Speake, personal communication) are presently being undertaken to determine whether an inadequacy of lipoprotein lipase activity *per se.*, or a cofactor deficiency (Apoprotein C2) is involved in embryo mortality (Connor, 1993; Noble and Speake, personal communication).

It is clear from the present investigations that there were obvious differences in the polyunsaturated fatty acid compositions in the phospholipids of the subcellular components between embryos from mature and young parent stock. These findings accord with previous observations (Noble, personal communication) in which a higher level of lipid unsaturation was observed in the yolk sac membrane and the livers of embryos from young parents compared with those from mature stock. It may be suggested from previous observations (Noble and Shand, 1985) that, in the present circumstances, embryos from the young parent stock may show some disturbances in their desaturation activities, that is, the conversion of C18 fatty acid precursors to long chain C20 and C22 products.

As already noted (see p133), changes in the polyunsaturated fatty acid levels of membranes or groups of membranes, may lead to alterations in fluidity, with consequential disturbances to a host of functions, vital to cell metabolism (Shand and Noble, 1981a; see Stubbs and Smith, 1984; Berlin *et al.*, 1989). In addition, modifications to membrane lipid composition may have consequential effects on receptor binding (Berlin *et al.*, 1989) and osmotic stability (Shand and Noble, 1981a). It is interesting to note that, with respect to a phylogenetically related species to the chicken, namely the alligator, under conditions of low tissue polyunsaturated fatty acid

concentrations, a significant reduction in hatchability has been observed (Noble, McCartney and Fergusson, 1992).

With respect to avians, studies during the final week of incubation in embryos from young and mature parent stock have shown distinct differences in the rate of hepatic lipid oxidation (Noble *et al.*, 1986b). In contrast to embryos from mature parents, the rates of oxidation in the livers of embryos from young parents, were significantly higher at day 15 of incubation (Noble *et al.*, 1986b).

Mammalian studies have shown that linoleic acid is very important for cardiac function; indeed a reduction in its levels has been shown to cause chaotic disturbances in cardiac rhythm (Abraham, Reimersma, Wood, Elton and Oliver, 1989: Sargent and Reimersma, 1990). Thus, it may be suggested that, in the embryos from both parental groups, increases in linoleic and arachidonic acid levels within the heart mitochondria are correlated with oxidation rates of the heart tissue as hatching time approaches. The reduced concentration of triglycerides in the mitochondria and microsomes of the heart in embryos from young parents, at the time of hatching, together with associated changes in fatty acid compositions, may indicate a significant reduction in the rate of lipid turnover.

Changes in the relative distributions of several other components in the yolk, some of which have particularly important aspects on lipid metabolism during the incubation period, have also been shown to be related to the age of the parent bird (Cunningham, Cotterill and Funk, 1960). Paramount amongst these factors are levels of zinc within the yolk whose intimate metabolic association with vitamin E metabolism would already have "knock on" effects on polyunsaturated fatty acid metabolism (Lu and Combs, 1988). In its own right, zinc is an important cofactor for adequate fatty acid

desaturase activity (see Cunnane, Huang, Horrobin and Davingnon, 1981), with consequential effects outlined above.

With respect to the trace elements, recent studies on the alligator have shown that, under conditions where selenium levels are reduced, hatachability showed a significant decrease (Noble *et al.*, 1992). Selenium is an important component of the antioxidant system, glutathione peroxidase (Rotruck *et al.*, 1973) and thus any reduction in selenium availability may also be expected to have a profound effect on polyunsaturated fatty acid metabolism.

In spite of the range of lipid compositional and metabolic differences observed presently and previously between embryos from mature and young parent stock, electron microscopic studies undertaken, have failed to show any obvious difference in structural morphology of the subcellular components. Lack of observable structural differences in themselves, does not preclude metabolic differences.

If severe lipid metabolic differences exist between the embryos from mature and young parent stock, then it is also essential to consider whether further complicatory features during incubation, are also present, with respect to respiratory gas exchange and water balance. A feature of the eggs from young parent stock was the observed extension in their hatching time, that is from 24 hours to 36 hours at hatching. Studies have revealed that eggs from young parent stock exhibit much lower porosity (Tullet and Noble, 1988a) and it may be suggested that there may be a limiting effect on embryo survival, brought about by an inability to maintain sufficient oxygen tension during incubation (Tullett and Burton, 1985) and in the blood returning to the embryo from the chorioallantois (Metcalfe, McCutcheon, Francisco, Metzenberg and

Welch, 1981). Although under normal circumstances the embryo is capable of tolerating high levels of carbon dioxide within the egg (Dawes, 1975), its ultimate level of accumulation is known to be involved in the hatching process by stimulating pipping of the eggshell (Visschedijk, 1968). It is generally accepted that adequacy of stimulus occurs when the carbon dioxide level in the air sac reaches about 5 per cent by volume (Tullet and Noble, 1988a) of the total air space gases. The combination of present and previous investigations on gross lipid movement into the embryo and subsequent lipid metabolism between embryos from mature and young parent stock, all so far show a reduction in the rate of utilisation of lipid substrate (Noble *et al.*, 1986b, Yafei and Noble, 1990). Investigations on air sac gases (Tullet and Noble, 1988a) and their compositional changes over the incubation period of such embryos, have shown that during the last week of incubation, carbon dioxide levels in the air sacs were significantly lower in embryos from young parent stock compared with those from mature parent stock (for example, 3.5 per cent carbon dioxide v/v compared with 5.2 per cent v/v in embryos from normal mature parent stock at day 19 of incubation).

Considerable controversy exists as to whether a lack of oxygen or an accumulation of carbon dioxide is the major feature with respect to embryo viability (Metcalfe *et al.*, 1981; Tullet and Burton, 1985; Tullet and Noble, 1988b; Sparks, personal communication). Clearly however, if carbon dioxide accumulation in the air sacs is a direct stimulus to pipping, then its lower level in eggs from young parent stock could be a causative factor in the non-emergence of the chick at the normally expected time. Most certainly, a greater recovery of viable chicks can be obtained if the hatching time is extended, but such a move would of course be unacceptable in commercial terms.

A certain amount of confusion still exists with respect to the porosity differences observed in the shells of eggs from mature and young parent stock. To date no structural differences have been confirmed in the matrix of the shell from eggs of young parents to suggest an alteration in calcium mobilisation during shell resorption (Sparks, personal communication). No skeletal abnormalities in addition, have been detected in the emergent chicks from young parents (Sparks and Burgess, personal communication).

4 The relationship between desmosterol and cholesterol in the brain.

Throughout the present investigations and in common with those observed previously, it is clear that free cholesterol plays an important role in the uptake and subsequent metabolism of yolk lipid by the embryo (Noble and Moore, 1967b; Noble and Connor, 1982; Noble *et al.*, 1984; see Noble, 1986, 1987b). With respect to cholesterol, its accumulation is particularly important within the brain and other nervous tissue (Connor *et al.*, 1969; Jain *et al.*, 1972; see Van den Berg, 1974; Wong and Lennarz, 1982: Noble and Cocchi, 1989a). The present observations on embryos from young parents have shown that within the brain, although amounts of cholesterol remained higher, they were accompanied by significant and relatively higher proportions of desmosterol. It has been shown that within the developing chick embryo, substituent sterols can replace cholesterol readily by dietary manipulation (Brown, Burgess and Wilson, 1962; Clarenburg, Chung and Wakefield, 1971; Singh *et al.*, 1972; see Naber, 1976). The results obtained in the present work on brain therefore, indicate an inability of yolk cholesterol to fully satisfy the metabolic requirements of the embryo and consequently an increase in demand made of a substituent sterol. With respect to this, it is interesting to note that in a most recent investigation on hatchability in eggs from young parent stock, overall chick yield in

eggs obtained from twenty-three to twenty-five week-old parents rose by 8.9 per cent under conditions where the cholesterol levels in the eggs were increased (Akinyemi, 1989; Noble, personal communication). From the survey literature there are indications that, when the levels of cholesterol have been deliberately enhanced within the eggs, egg production and hatchability rates have been considerably reduced (Singh *et al.*, 1972; see Naber, 1976).

5 Vitamin E (alpha tocopherol) and polyunsaturated fatty acid metabolism.

As previous studies have shown there is a considerable increase in the proportion of polyunsaturated fatty acids in the yolk sac membrane and liver of the chick embryo during the final week of incubation (Noble and Moore, 1964, 1965b, 1967a,c). As demonstrated in the present investigations, this is translated into effects at the subcellular level. The particularly high content of polyunsaturated fatty acids in the tissues would make them increasingly susceptible to peroxidation and the formation of hydroperoxides, free radicals and other toxic products, destructive to the cell (see Mead, 1976; see Esterbauer, 1982). Studies at the subcellular level have shown that lipid peroxidation can give rise to mitochondrial swelling (Hunter *et al.*, 1963, 1964a) and the efflux of matrix constituents from the swollen organelles (Hunter *et al.*, 1963, 1964a) and also affect ion conductivity of the intrinsic bilayer (Ohki and Goldup, 1968). Protection of polyunsaturated fatty acids via antioxidants is therefore of vital importance. The ability of an antioxidant to destroy the highly reactive radicals maintains the structural integrity of the cell and prevents the depletion of the required nutrients or metabolites (see Pryor, 1976; see Halliwell and Gutteridge, 1985).

The most prominent naturally occurring antioxidant is vitamin E; its function as a free radical scavenger is well established (see Tappel, 1972; Hoeskra, 1975; see McCay and King, 1980). Vitamin E is particularly important as an inter- and intracellular antioxidant where it stabilises the ingested polyunsaturated fatty acids and the synthesis and degradation of lipids. In addition, the physicochemical interaction of α-tocopherol with certain fatty acids may maintain membrane stability (Diplock and Lucy, 1973; see Frankel, 1982, 1985), as well as removing abnormal lipids such as the polymerised polyunsaturated fatty acids (Hayes *et al.*, 1969; see Frankel, 1982, 1985), which may be implicated in the regulatory process.

An important consideration in the nutrition of the breeding hen is the need to provide an adequate carry-over of vitamin E, into the egg and subsequently into the hatching chick. An association between vitamin E levels within the egg and embryo mortality or vitamin E deficiency in the young chick is well established (Adamstone, 1931, Almquist, 1956; Singsen *et al.*, 1954). The relationship between dietary vitamin E and levels within the egg is not a direct one but does depend upon a range of metabolic features involving transport mechanisms (see Whitehead and Portsmouth, 1989). Thus, studies have shown the involvement of vitamin E with a range of specific binding proteins, both within the hen and within the yolk (see Whitehead and Portsmouth, 1989).

In the present investigations the levels of α-tocopherol within the yolk initially were in accordance with previous measurements. In overall terms, it has been shown that there is a direct linear relationship between the α-tocopherol content of the liver at hatching and the initial α-tocopherol content of the yolk (Bartov, Budowski and Bornstein, 1963). As shown in the present investigations, the accumulation of α-tocopherol by the embryo liver is far from linear over the incubation period but it is

particularly marked during the final period of incubation, commensurate with overall yolk lipid movement (Noble and Moore, 1967a,c).

Clearly by day 19 of incubation, under normal circumstances, present results would suggest that adequate protection of the polyunsaturated fatty acids in the liver is provided by the very high levels of α-tocopherol. However, what is also obvious from the results, is that the extensive accumulation of α-tocopherol within the liver at day 19 of incubation occurs well after the time when lipid unsaturation and their requirements for protection against oxidation are most extreme. At the time of highest lipid unsaturation, namely at day 15 of incubation, α-tocopherol levels were still extremely low. The delayed accumulation of α-tocopherol in the liver during the hatching process, relative to the polyunsaturated fatty acid levels of the tissue, may therefore, under certain circumstances, pose an element of risk to the survival of the embryo. This is particularly so if account is taken of the accumulation of double bonds in the developing embryo tissues, rather than accumulation of unsaturated fatty acid molecules.

Although differences were observed in the tissue levels of both α-tocopherol and polyunsaturated fatty acids between embryos from mature and young parents, in terms of ratios of α-tocopherol levels to numbers of double bonds, there were no differences. However, in embryos from young parent stock, a very much higher proportion of lipid remains associated with the yolk sac membrane. The possibility exists therefore, that this large proportion of unsaturated material may be a factor in viability due to the independent protection as some transport of α-tocopherol into the tissues has occurred, leaving the yolk sac membrane exposed.

A significant feature of the present results is the high level of polyunsaturated fatty acids, in particular docosahexaenoic acid within the brain of the developing embryo from day 13 of incubation, but the virtually complete absence of α-tocopherol. Clearly, an alternative protective mechanism must exist and recent evidence (MacPherson, personal communication) has shown an enhanced role for glutathione peroxidase. Selenium is an important component of glutathione peroxidase and it has been shown (MacPherson, personal communication) that selenium levels within the brain are increased rapidly from day 10 of incubation onwards, commensurate with brain function (Marco, Alejandre, Gonzalez-Pacanowska, Segovia, and García-Peregrin, 1985; Bordoni *et al.*, 1986).

From present observations it would appear that once within the neonatal tissues, the α-tocopherol is highly retained, but clearly not by the liver or heart. The suggestion is therefore that, following hatching, a rapid redistribution of α-tocopherol occurs amongst the tissues in general, possibly associated with a vital role in the maintenance activity of the immune system (Tengerdy, Heinzerling and Nockels, 1972; Tengerdy and Brown, 1977; see Sheffy and Schultz, 1979; Tengerdy *et al.*, 1981).

6 General Conclusions.

Lipid assimilation during the last seven days of embryonic development is characterised by extensive and in many instances, distinctive changes in lipid and fatty acid compositions, both at the cellular and subcellular levels. The changes in lipid and fatty acid compositions are specific for each tissue and organelle fraction and clearly relate to the metabolic role of that tissue throughout embryonic and post-hatch development. It is consequently true to say that in all tissue fractions the relationship between major lipid classes will change in accordance with the tissue requirements so as to maintain the correct balance for efficient function.

It has been suggested therefore, that such changes do have an effect on chick viability. In certain instances, the present observations would recommend a tangible means of approaching what is a highly practical problem. Most certainly, intensive production systems have highlighted the pressures being imposed on certain metabolic mechanisms; within these, lipid mechanisms are undoubtedly of importance.

7 Future work.

The present analytical investigations throughout the embryonic and post-hatch periods have revealed changes in the lipid and fatty acid compositions, particularly in the subcellular fractions of the liver and heart. Subsequent alterations to the biochemical conformation and membrane permeability of the cell membranes as a result of these changes, can only be speculated upon. Techniques to assess membrane fluidity could be used to confirm any changes in membrane permeability which may occur throughout development. Previous studies have shown that membrane fatty acid compositions can be modified by alterations in diet (see Introduction; Sections 8.1 and 8.2 of the Literature Review). Modifications to the diet of the parent bird or chick therefore, may provide some insight into the mechanism by which lipid and fatty acid levels change to compensate any alterations in membrane fluidity.

The type of phospholipid dominating the subcellular fractions of the liver, heart and brain throughout the embryonic and post-hatch periods has not been included in these present studies. Separation and analyses of the phospholipid classes would identify those phospholipids, which showed the most obvious changes in fatty acid composition throughout development.

A comparison between offspring from mature and young parents revealed differences throughout development in the lipid and fatty acid compositions of the liver and heart subcellular fractions. Previous studies have confirmed differences in the lipid composition of the yolk and tissues of embryos from mature and young parents (see Section 7 of the Literature Review). Work is presently ongoing (Noble, Speake *et al.*) to assess whether any differences in the activity of lipoprotein lipase and of several

other enzymes involved in cholesterol esterification, could account for the subsequent metabolic differences observed between the two parental groups. From these studies, it may be necessary to modify the diet of the young parent for example, in such a way that the embryo obtains the necessary nutrients for normal development and hatchability rates are increased.

The importance of vitamins E and C as antioxidants and their role in metabolism and immune development has been well documented (see Section 9 of the Literature Review). Knowledge on vitamin nutrition has much increased over the years and this has brought with it changes in feeding practice. Current research suggests that this process will continue. More specific information would be useful on the roles of individual vitamins, particularly E and C, in relation to both heat and transport stress. Alleviation of the latter stress in particular could substantially improve bird welfare and broiler processing yield.

Following hatching, the rapid loss of α-tocopherol from the livers of the chick is interesting. Since there was no indication from chromatography studies to suggest that α-tocopherol breakdown occurred, it can be inferred that it was transported elsewhere. Studies using selective radioactive labelling could establish the pathway of α-tocopherol transport following its assimilation into the embryonic liver.

The results showed that there was a lack of synchrony between optimal levels of α-tocopherol and maximum levels of polyunsaturated fatty acids, suggesting a possible risk to the survival of the developing embryo. Measurement of peroxidation levels in the liver may provide some indication as to metabolic stresses imposed on embryos at the time of hatching.

The present investigations have confirmed low levels of α-tocopherol in the brain tissue. In an environment which is rich in polyunsaturated fatty acids, it is clear that some other mechanism must exist in the brain by which lipid peroxidation is prevented. Research is continuing in this field (MacPherson, personal communication).

CHAPTER 6
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CHAPTER 7

RESULTS

(TABLES)

days after hatch	embryos and chicks from mature parents[a]	embryos and chicks from young parents[b]
13d	18.72 ± 0.97	16.24 ± 1.22
19d	99.81 ± 3.42***	62.42 ± 2.38
days after hatch:		
1DO	110.85 ± 1.42***	80.32 ± 1.44

The number of replicates used for all analyses was 4.
[a] = thirty-seven to forty weeks of age.
[b] = twenty-three to twenty-four weeks of age.
Significance of difference between corresponding embryos/ chicks from mature and young parents: ***=$P<0.01$

Table 5 Concentrations of total lipid (mean weight in milligrams ± SE of the total lipid) associated with the livers of embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d	0.91 ±0.04	1.05 ±0.03	0.90 ±0.08	3.90 ±0.33	2.66 ±0.04	0.71 ±0.05
16d	2.17 ±0.21	1.63 ±0.08	1.90 ±0.04	NA[c]	NA	NA
19d	1.74 ±0.06	2.00 ±0.39	0.78 ±0.14	2.82 ±0.10	2.03 ±0.19	0.68 ±0.02
days after hatch:						
1DO	1.40 ±0.13	1.43 ±0.13	0.46 ±0.14	2.40 ±0.14	2.16 ±0.36	0.50 ±0.08
6DO	1.74 ±0.28	1.09 ±0.14	0.44 ±0.01	3.25 ±0.50	1.21 ±0.27	0.77 ±0.38
12DO	1.86 ±0.14	0.87 ±0.05	0.66 ±0.01	2.76 ±0.16	1.77 ±0.13	0.67 ±0.24

The number of replicates used for analysis was- embryos from mature parents 5; embryos from young parents 4; chicks from mature and young parents 4.

[a] = thirty-seven to forty weeks of age.

[b] = twenty-three to twenty-four weeks of age.

[c] = no samples available due to high embryo mortality.

Table 6 Total concentrations of lipid (mean weight in milligrams ± SE of the total lipid) per gram of tissue associated with the mitochondria, microsomes and supernatant of the livers of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d v 19d	***	NS	NS	*	*	NS
19d v 1DO	NS	NS	NS	NS	NS	NS
1DO v 6DO	NS	NS	NS	NS	NS	NS
1DO v 12DO	NS	**	NS	NS	NS	NS

The results refer to the data shown in Table 6.
Significance of difference: *=P<0.05 **=P<0.01 ***=P<0.001
NS= Not Significant
[a,b]- notations as in Table 6.

Table 7a The levels of significance for the changes in lipid concentration in the mitochondria, microsomes and supernatant of the liver during embryonic and post-hatch development.

	mitochondria	microsomes	supernatant
13d v 13d	***	***	NS
19d v 19d	***	NS	NS
1DO v 1DO	**	NS	NS
6DO v 6DO	*	NS	NS
12DO v 12DO	**	***	NS

The results refer to the data shown in Table 6.
Significance of difference- notations as in Table 7a.

Table 7b The levels of significance for the changes in lipid concentration in the mitochondria, microsomes and supernatant of the liver between embryos and post-hatch chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d	0.61 ±0.07	0.55 ±0.06	0.10 ±0.01	0.98 ±0.06	0.59 ±0.08	0.12 ±0.04
16d	0.42 ±0.05	0.56 ±0.08	0.24 ±0.06	NA[c]	NA	NA
19d	0.46 ±0.04	0.42 ±0.03	0.20 ±0.04	0.73 ±0.04	0.54 ±0.04	0.10 ±0.02
days after hatch:						
1DO	0.48 ±0.10	0.56 ±0.04	0.18 ±0.02	0.95 ±0.17	0.48 ±0.04	0.16 ±0.02
6DO	0.40 ±0.03	0.28 ±0.04	0.10 ±0.04	0.90 ±0.20	0.57 ±0.11	0.18 ±0.06
12DO	0.43 ±0.12	0.21 ±0.04	0.11 ±0.05	0.89 ±0.18	0.54 ±0.03	0.24 ±0.01

The number of replicates used for all analyses was 4.
[a,b,c] - notations as in Table 6.

Table 8 Total concentrations of lipid (mean weight in milligrams ± SE of the total lipid) per gram of tissue associated with the mitochondria, microsomes and supernatant of the hearts of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d v 19d	NS	NS	NS	*	NS	NS
19d v 1DO	NS	*	NS	NS	NS	NS
1DO v 6DO	NS	**	NS	NS	NS	NS
1DO v 12DO	NS	***	NS	NS	NS	*

The results refer to the data shown in Table 8.
Significance of difference- notations as in Table 7a.
[a,b]- notations as in Table 6

Table 9a The levels of significance for the changes in lipid concentration in the mitochondria, microsomes and supernatant of the heart during embryonic and post-hatch development.

	mitochondria	microsomes	supernatant
13d v 13d	**	NS	NS
19d v 19d	**	NS	NS
1DO v 1DO	NS	NS	NS
6DO v 6DO	*	*	NS
12DO v 12DO	NS	***	*

The results refer to the data shown in Table 8.
Significance of difference- notations as in Table 7a.

Table 9b The levels of significance for the changes in lipid concentration in the mitochondria, microsomes and supernatant of the heart between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d	2.68 ±0.24	1.14 ±0.01	0.21 ±0.01	4.18 ±0.16	1.67 ±0.22	0.18 ±0.05
16d	2.24 ±0.16	2.25 ±0.42	0.19 ±0.05	NA[c]	NA	NA
19d	2.97 ±0.14	1.75 ±0.21	0.25 ±0.05	4.21 ±0.36	2.24 ±0.08	0.15 ±0.01
days after hatch:						
1DO	1.70 ±0.25	1.13 ±0.07	0.24 ±0.06	3.57 ±0.39	1.62 ±0.22	0.16 ±0.02
6DO	2.43 ±0.15	0.79 ±0.10	0.14 ±0.05	4.24 ±0.51	1.08 ±0.88	0.17 ±0.03
12DO	2.36 ±0.24	0.94 ±0.10	0.08 ±0.04	2.90 ±0.15	1.00 ±0.13	0.13 ±0.04

The number of replicates used for all analyses was 4.
[a,b,c]- notations as in Table 6.

Table 10 Total concentrations of lipid (mean weight in milligrams ± SE of the total lipid) per gram of tissue associated with the mitochondria, microsomes and supernatant of the brains of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d v 19d	NS	*	NS	NS	NS	NS
19d v 1DO	**	*	NS	NS	*	NS
1DO v 6DO	*	*	NS	NS	NS	NS
1DO v 12DO	NS	NS	NS	NS	NS	NS

The results refer to the data shown in Table 10.
Significance of difference- notations as in Table 7a.
[a,b]- notations as in Table 6.

Table 11a The levels of significance for the changes in lipid concentration in the mitochondria, microsomes and supernatant of the brain during embryonic and post-hatch development.

	mitochondria	microsomes	supernatant
13d v 13d	**	NS	NS
19d v 19d	*	NS	NS
1DO v 1DO	**	NS	NS
6DO v 6DO	*	NS	NS
12DO v 12DO	NS	NS	NS

The results refer to the data shown in Table 10.
Significance of difference- notations as in Table 7a.

Table 11b The levels of significance for the changes in lipid concentration in the mitochondria, microsomes and supernatant of the brain between embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d v 19d	NS	*	NS	NS	NS	NS
19d v 1DO	**	*	NS	NS	*	NS
1DO v 6DO	*	*	NS	NS	NS	NS
1DO v 12DO	NS	NS	NS	NS	NS	NS

The results refer to the data shown in Table 10.
Significance of difference- notations as in Table 7a.
[a,b]- notations as in Table 6.

Table 11a The levels of significance for the changes in lipid concentration in the mitochondria, microsomes and supernatant of the brain during embryonic and post-hatch development.

	mitochondria	microsomes	supernatant
13d v 13d	**	NS	NS
19d v 19d	*	NS	NS
1DO v 1DO	**	NS	NS
6DO v 6DO	*	NS	NS
12DO v 12DO	NS	NS	NS

The results refer to the data shown in Table 10.
Significance of difference- notations as in Table 7a.

Table 11b The levels of significance for the changes in lipid concentration in the mitochondria, microsomes and supernatant of the brain between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d	31.8	36.7	31.5	53.8	36.5	9.7
16d	38.1	28.6	33.3	NA[c]	NA	NA
19d	38.5	44.2	17.3	51.0	36.7	12.3
days after hatch:						
1DO	42.6	43.5	14.0	47.4	42.7	9.9
6DO	53.2	33.3	13.5	62.1	23.1	14.7
12DO	54.7	25.7	19.5	53.1	34.0	12.9

Number of replicates as for Table 6.
[a,b,c]- notations as in Table 6.

Table 12 The percentage distribution of total lipid between the mitochondria, microsomes and supernatant of the livers of embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d	48.5	43.6	7.9	58.0	34.9	7.1
16d	34.4	45.9	19.7	NA[c]	NA	NA
19d	42.6	38.9	18.5	53.3	39.4	7.3
days after hatch:						
1DO	39.34	45.90	14.75	59.75	30.19	10.06
6DO	51.28	35.90	12.82	54.54	34.54	10.91
12DO	57.33	28.00	14.67	53.29	32.34	14.37

Number of replicates as for Table 8.
[a,b,c]- notations as in Table 6.

Table 13 The percentage distribution of total lipid between the mitochondria, microsomes and supernatant of the hearts of embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
13d	66.5	28.3	5.2	69.3	27.7	3.0
16d	47.9	48.1	4.1	NA[c]	NA	NA
19d	59.8	35.2	5.0	63.8	33.9	2.3
days after hatch:						
1DO	55.4	36.8	7.8	66.7	30.3	3.0
6DO	72.3	23.5	4.2	77.2	19.7	3.1
12DO	69.8	27.8	2.4	72.0	24.9	3.2

Number of replicates as for Table 10.
[a,b,c]- notations as in Table 6.

Table 14 The percentage distribution of total lipid between the mitochondria, microsomes and supernatant of the brains of embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d	70.03 ±0.83	14.15 ±0.42	4.50 ±0.52	6.64 ±0.32	4.68 ±0.32	73.13 ±0.94	11.37 ±0.16	2.26 ±0.08	7.24 ±0.06	6.04 ±1.11
16d	69.18 ±2.43	13.29 ±0.60	2.75 ±0.28	6.48 ±0.61	8.30 ±1.75	NA[d]	NA	NA	NA	NA
19d	74.67 ±2.44	14.10 ±1.19	2.38 ±0.44	3.67 ±0.72	5.18 ±1.42	81.83 ±0.87	9.18 ±0.32	1.34 ±0.20	2.96 ±0.83	4.68 ±0.72
days after hatch:										
1DO	72.04 ±0.66	12.67 ±0.39	2.10 ±0.63	5.14 ±0.38	8.02 ±0.67	81.06 ±2.41	5.32 ±1.25	1.62 ±0.35	5.81 ±0.91	1.46 ±0.16
6DO	67.48 ±2.09	10.72 ±1.50	3.20 ±0.49	16.38 ±0.82	2.23 ±0.17	76.08 ±2.21	7.40 ±0.82	1.84 ±0.67	13.81 ±0.85	1.49 ±0.24
12DO	66.62 ±1.65	9.90 ±0.92	2.21 ±0.25	19.63 ±1.35	1.63 ±0.81	73.39 ±0.73	10.31 ±0.55	2.47 ±0.21	12.75 ±0.24	1.1 ±0.21

The number of replicates used for analysis was- embryos from mature parents 5; embryos from young parents 4; chicks from mature and young parents 4.

[a]= thirty-seven to forty weeks of age.
[b]= twenty-three to twenty-four weeks of age.
[c]= PL- phospholipids FFA- free fatty acids CE- cholesteryl esters FC- free cholesterol TG- triglycerides
[d]= no samples available due to high embryo mortality.

Table 15 The relative concentrations (weight percentages ± SE of the lipid) of the major lipid components in the mitochondria of the livers of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d v 19d	NS	NS	*	**	NS	***	***	**	***	NS
19d v 1DO	NS	NS	NS	NS	NS	NS	*	NS	*	**
1DO v 6DO	NS	NS	NS	***	***	NS	NS	NS	***	NS
1DO v 12DO	*	*	NS	***	***	*	*	NS	***	NS

The results refer to the data shown in Table 15.
[a,b,c]- notations as in Table 15.
Significance of difference: *=P<0.05 **=P<0.01 ***=P<0.001
NS= Not Significant

Table 16a The levels of significance for the changes in relative concentrations of the major lipid components in the mitochondria of the liver during embryonic and post-hatch development.

	mitochondria				
	PL[a]	FC[a]	FFA[a]	TG[a]	CE[a]
13d v 13d	*	***	**	NS	NS
19d v 19d	*	**	NS	NS	NS
1DO v 1DO	*	**	NS	NS	***
6DO v 6DO	*	NS	NS	NS	*
12DO v 12DO	**	NS	NS	**	NS

The results refer to the data shown in Table 15.
[a]= PL- phospholipids FFA- free fatty acids CE- cholesteryl esters
FC- free cholesterol TG- triglycerides
Significance of difference- notations as in Table 16a.

Table 16b The levels of significance for the changes in the relative concentrations of the major lipid components in the mitochondria of the liver between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d	61.30 ±2.02	24.32 ±2.71	4.15 ±0.23	6.76 ±1.67	3.46 ±0.37	62.24 ±2.39	25.24 ±3.12	2.17 ±0.32	7.24 ±0.86	3.01 ±0.39
16d	66.60 ±1.12	19.72 ±1.65	4.83 ±1.04	3.94 ±0.15	4.91 ±0.95	NA[d]	NA	NA	NA	NA
19d	67.27 ±1.94	19.33 ±0.80	2.86 ±0.13	6.18 ±1.16	4.36 ±0.24	68.70 ±2.38	21.21 ±2.13	1.07 ±0.12	3.27 ±0.28	1.16 ±0.24
days after hatch:										
1DO	62.80 ±2.02	14.21 ±2.29	3.01 ±0.52	14.99 ±1.04	4.99 ±1.15	73.74 ±2.16	11.49 ±0.80	3.91 ±1.07	6.66 ±2.37	4.20 ±0.81
6DO	60.91 ±3.34	13.94 ±0.19	2.87 ±0.37	21.88 ±3.10	1.90 ±0.54	70.62 ±3.92	12.31 ±1.76	2.34 ±2.34	13.92 ±2.03	0.80 ±0.22
12DO	61.12 ±3.16	10.15 ±0.43	3.26 ±0.80	22.92 ±1.60	1.39 ±0.45	69.64 ±2.42	9.94 ±2.18	1.92 ±0.15	17.74 ±1.31	0.76 ±0.29

The number of replicates used for all analyses was 4.
[a,b,c,d]- notations as in Table 15.

Table 17 The relative concentrations (weight percentages ± SE of the lipid) of the major lipid components in the mitochondria of the hearts of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d v 19d	NS	NS	**	NS	NS	NS	NS	*	**	**
19d v 1DO	NS	NS	NS	**	NS	NS	**	*	NS	*
1DO v 6DO	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
1DO v 12DO	NS	NS	NS	**	*	NS	NS	NS	**	**

The results refer to the data shown in Table 17.
[a,b,c]- notations as in Table 15.
Significance of difference- notations as in Table 16a.

Table 18a The levels of significance for the changes in relative concentrations of the major lipid components in the mitochondria of the heart during embryonic and post-hatch development.

	mitochondria				
	PL[a]	FC[a]	FFA[a]	TG[a]	CE[a]
13d v 13d	NS	NS	**	NS	NS
19d v 19d	NS	NS	***	NS	***
1DO v 1DO	*	NS	NS	*	NS
6DO v 6DO	NS	NS	NS	NS	NS
12DO v 12DO	NS	NS	NS	*	NS

The results refer to the data shown in Table 17.
[a]- notation as in Table 16b.
Significance of difference- notations as in Table 16a.

Table 18b The levels of significance for the changes in relative concentrations of the major lipid components in the mitochondria of the heart between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d	69.60 ±0.99	28.52 ±0.32	0.34 ±0.26	1.00 ±0.43	0.54 ±0.19	71.30 ±1.20	27.08 ±1.20	0.49 ±0.32	0.83 ±0.36	0.30 ±0.21
16d	69.83 ±1.99	25.99 ±2.27	1.96 ±0.17	1.21 ±0.17	0.78 ±1.20	NA[d]	NA	NA	NA	NA
19d	63.39 ±2.06	24.86 ±1.04	2.09 ±0.75	2.15 ±0.75	1.51 ±0.62	73.15 ±2.11	24.71 ±1.94	0.60 ±0.10	0.98 ±0.12	0.56 ±0.02
days after hatch:										
1DO	68.31 ±3.00	23.68 ±1.59	1.52 ±0.27	4.72 ±1.83	1.76 ±0.41	66.78 ±0.99	29.31 ±1.76	1.35 ±0.23	1.56 ±0.44	1.38 ±0.51
6DO	60.64 ±0.85	35.71 ±1.26	1.83 ±0.01	1.82 ±0.83	3.05 ±0.73	61.82 ±1.48	33.83 ±1.08	1.62 ±0.33	1.26 ±0.34	0.81 ±0.16
12DO	67.58 ±0.99	29.72 ±1.09	0.77 ±0.47	1.68 ±0.41	0.25 ±0.14	63.82 ±0.72	31.78 ±0.72	1.04 ±0.34	2.64 ±0.75	0.46 ±0.04

The number of replicates used for all analyses was 4.
[a,b,c,d]- notations as in Table 15.

Table 19 The relative concentrations (weight percentages ± SE of the lipid) of the major lipid components in the mitochondria of the brains of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d v 19d	*	*	NS	NS	NS	NS	NS	NS	NS	NS
19d v 1DO	NS	NS	NS	NS	NS	*	NS	*	NS	NS
1DO v 6DO	*	**	NS	NS	NS	*	NS	NS	NS	NS
1DO v 12DO	NS	*	NS	NS	*	NS	NS	NS	NS	NS

The results refer to the data shown in Table 19.
[a,b,c]- notations as in Table 15.
Significance of difference- notations as in Table 16a.

Table 20a The levels of significance for the changes in relative concentrations of the major lipid components in the mitochondria of the brain during embryonic and post-hatch development.

	mitochondria				
	PL[a]	FC[a]	FFA[a]	TG[a]	CE[a]
13d v 13d	NS	NS	NS	NS	NS
19d v 19d	*	NS	NS	NS	NS
1DO v 1DO	NS	NS	NS	NS	NS
6DO v 6DO	NS	NS	NS	NS	*
12DO v 12DO	*	NS	NS	NS	NS

The results refer to the data shown in Table 19.
[a]- notation as in Table 16b.
Significance of difference- notations as in Table 16a.

Table 20b The levels of significance for the changes in relative concentrations of the major lipid components in the mitochondria of the brain between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d	55.51 ±2.37	31.22 ±2.54	2.58 ±0.52	6.96 ±0.06	3.72 ±0.40	55.12 ±1.64	26.84 ±3.66	4.72 ±1.88	8.84 ±1.36	4.58 ±1.22
16d	61.17 ±1.94	19.13 ±1.11	4.03 ±0.21	8.26 ±0.58	7.40 ±1.35	NA[d]	NA	NA	NA	NA
19d	62.84 ±0.74	22.73 ±1.51	2.29 ±0.45	4.22 ±0.25	7.92 ±0.99	65.49 ±2.93	18.39 ±1.18	1.42 ±0.09	4.01 ±0.36	10.69 ±2.19
days after hatch:										
1DO	70.14 ±0.64	16.38 ±0.91	2.13 ±0.18	6.72 ±0.46	4.63 ±0.18	71.19 ±2.01	12.73 ±0.70	2.47 ±0.10	6.13 ±0.40	6.98 ±1.85
6DO	57.14 ±1.91	15.46 ±1.73	3.34 ±0.28	18.69 ±0.89	5.18 ±1.22	63.20 ±1.51	16.90 ±1.71	3.34 ±0.35	14.22 ±0.54	2.34 ±0.12
12DO	58.45 ±1.56	17.84 ±1.71	4.53 ±0.96	17.65 ±1.61	1.53 ±0.24	64.77 ±1.26	17.55 ±1.05	3.13 ±0.17	13.38 ±1.36	1.15 ±0.04

Number of replicates as for Table 15.
[a,b,c,d]- notations as in Table 15.

Table 21 The relative concentrations (weight percentages ± SE of the lipid) of the major lipid components in the microsomes of the livers of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d v 19d	*	*	NS	***	**	*	NS	NS	*	NS
19d v 1DO	***	**	NS	**	*	NS	**	***	**	NS
1DO v 6DO	***	NS	*	***	NS	*	NS	NS	***	*
1DO v 12DO	***	NS	*	***	***	*	**	*	**	*

The results refer to the data shown in Table 21.
[a,b,c]- notations as in Table 15.
Significance of difference- notations as in Table 16a.

Table 22a The levels of significance for the changes in relative concentrations of the major lipid components in the microsomes of the liver during embryonic and post-hatch development.

	microsomes				
	PL[a]	FC[a]	FFA[a]	TG[a]	CE[a]
13d v 13d	NS	NS	NS	NS	NS
19d v 19d	NS	NS	NS	NS	NS
1DO v 1DO	NS	*	NS	NS	NS
6DO v 6DO	*	NS	NS	**	NS
12DO v 12DO	*	NS	NS	NS	NS

The results refer to the data shown in Table 21.
[a]- notation as in Table 16b.
Significance of difference- notations as in Table 16a.

Table 22b The levels of significance for the changes in relative concentrations of the major lipid components in the microsomes of the liver between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d	56.64 ±5.16	31.78 ±3.32	4.98 ±0.37	3.47 ±0.66	3.12 ±0.33	57.62 ±3.12	34.24 ±2.36	2.21 ±0.12	3.00 ±0.42	2.93 ±0.34
16d	56.36 ±1.42	32.43 ±1.28	4.07 ±0.89	3.32 ±0.14	3.82 ±0.51	NA[d]	NA	NA	NA	NA
19d	62.45 ±3.02	26.96 ±3.11	3.18 ±0.26	4.49 ±0.23	2.86 ±0.42	54.27 ±3.15	37.15 ±1.86	3.07 ±0.40	3.28 ±0.58	2.21 ±0.88
days after hatch:										
1DO	58.31 ±2.58	15.35 ±1.35	4.31 ±0.46	18.34 ±0.92	3.63 ±0.30	65.26 ±1.64	21.46 ±2.26	4.10 ±0.67	5.77 ±1.01	3.41 ±0.43
6DO	51.00 ±1.46	26.14 ±3.88	4.08 ±0.46	15.34 ±2.84	2.28 ±0.31	49.66 ±4.96	19.66 ±2.65	2.36 ±0.32	21.09 ±2.11	1.09 ±0.22
12DO	60.65 ±2.71	21.04 ±2.50	2.41 ±0.48	14.80 ±2.21	1.07 ±0.48	49.97 ±2.88	26.14 ±2.44	2.60 ±0.14	20.74 ±0.60	0.64 ±0.13

Number of replicates as for Table 17.
[a,b,c,d]- notations as in Table 15.

Table 23 The relative concentrations (weight percentages ± SE of the lipid) of the major lipid components in the microsomes of the hearts of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d v 19d	NS	NS	**	NS	NS	NS	NS	NS	NS	NS
19d v 1DO	NS	*	NS	***	NS	*	**	NS	NS	NS
1DO v 6DO	NS	*	NS	NS	*	*	NS	NS	***	**
1DO v 12DO	NS	NS	*	NS	**	**	NS	NS	***	***

The results refer to the data shown in Table 23.
[a,b,c]- notations as in Table 15.
Significance of difference- notations as in Table 16a.

Table 24a The levels of significance for the changes in relative concentrations of the major lipid components in the microsomes of the heart during embryonic and post-hatch development.

	microsomes				
	PL[a]	FC[a]	FFA[a]	TG[a]	CE[a]
13d v 13d	NS	NS	***	NS	NS
19d v 19d	NS	*	NS	NS	NS
1DO v 1DO	NS	NS	NS	***	NS
6DO v 6DO	NS	NS	*	NS	*
12DO v 12DO	*	NS	NS	*	NS

The results refer to the data shown in Table 23.
[a]- notation as in Table 16b.
Significance of difference- notations as in Table 16a.

Table 24b The levels of significance for the changes in relative concentrations of the major lipid components in the microsomes of the heart between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d	66.70 ±0.89	29.40 ±0.56	1.33 ±0.12	1.02 ±0.22	0.84 ±0.28	62.24 ±1.62	33.10 ±1.23	1.24 ±0.32	1.92 ±0.34	1.5 ±0.42
16d	71.11 ±1.78	25.80 ±1.36	1.41 ±0.29	1.11 ±0.36	0.51 ±0.24	NA[d]	NA	NA	NA	NA
19d	69.55 ±2.76	25.26 ±3.24	1.64 ±0.09	2.61 ±0.49	0.93 ±0.23	58.18 ±1.65	39.21 ±1.53	1.13 ±0.15	0.84 ±0.15	0.63 ±0.10
days after hatch:										
1DO	64.32 ±2.14	29.24 ±1.10	1.50 ±0.53	3.88 ±0.68	1.06 ±0.38	65.79 ±2.80	31.76 ±2.64	1.40 ±0.10	1.14 ±0.28	0.66 ±0.12
6DO	56.18 ±1.51	36.01 ±0.95	2.89 ±0.95	3.18 ±0.69	1.60 ±0.64	63.24 ±1.27	29.40 ±1.37	1.58 ±0.36	2.13 ±0.23	3.09 ±1.46
12DO	65.93 ±2.15	28.54 ±2.38	1.71 ±0.44	2.83 ±0.60	0.99 ±0.33	65.27 ±3.43	27.68 ±2.66	2.34 ±0.30	3.40 ±0.58	1.32 ±0.28

Number of replicates as for Table 19.
[a,b,c,d]- notations as in Table 15.

Table 25 The relative concentrations (weight percentages ± SE of the lipid) of the major lipid components in the microsomes of the brains of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d v 19d	NS	NS	NS	*	NS	NS	*	NS	NS	NS
19d v 1DO	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
1DO v 6DO	*	**	NS	NS	NS	NS	NS	NS	NS	NS
1DO v 12DO	NS	NS	NS	NS	NS	NS	NS	*	*	NS

The results refer to the data shown in Table 25.
[a,b,c]- notations as in Table 15.
Significance of difference- notations as in Table 16a.

Table 26a The levels of significance for the changes in relative concentrations of the major lipid components in the microsomes of the brain during embryonic and post-hatch development.

	microsomes				
	PL[a]	FC[a]	FFA[a]	TG[a]	CE[a]
13d v 13d	NS	*	NS	NS	NS
19d v 19d	*	**	NS	*	NS
1DO v 1DO	NS	NS	NS	**	NS
6DO v 6DO	*	**	NS	NS	NS
12DO v 12DO	NS	NS	NS	NS	NS

The results refer to the data shown in Table 25.
[a]- notation as in Table 16b.
Significance of difference- notations as in Table 16a.

Table 26b The levels of significance for the changes in relative concentrations of the major lipid components in the microsomes of the brain between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d	12.68 ±1.42	20.44 ±1.12	3.54 ±0.14	25.18 ±0.36	38.36 ±3.04	12.72 ±1.34	21.45 ±3.21	4.01 ±0.92	22.46 ±1.23	39.36 ±2.96
16d	8.31 ±1.51	8.70 ±0.13	0.59 ±0.58	11.52 ±0.13	70.87 ±1.77	NA[d]	NA	NA	NA	NA
19d	14.90 ±2.50	11.76 ±2.70	1.92 ±0.40	9.44 ±1.96	62.00 ±7.56	14.97 ±1.56	6.24 ±1.91	1.01 ±0.26	10.88 ±2.58	66.89 ±2.78
days after hatch:										
1DO	18.82 ±2.70	11.77 ±0.71	2.06 ±0.41	11.90 ±2.26	55.43 ±3.42	20.12 ±2.80	12.73 ±1.14	1.68 ±0.60	9.56 ±0.91	55.90 ±3.18
6DO	15.44 ±5.88	11.92 ±2.24	3.04 ±0.70	52.22 ±5.73	17.38 ±1.38	15.58 ±5.74	6.01 ±5.88	1.87 ±1.05	65.72 ±7.02	10.82 ±4.23
12DO	15.29 ±3.64	5.74 ±0.52	4.01 ±0.06	71.92 ±3.96	3.04 ±0.92	21.67 ±2.55	4.89 ±2.77	2.22 ±0.26	65.82 ±7.32	5.39 ±2.74

Number of replicates as for Table 15.
[a,b,c,d]- notations as in Table 15.

Table 27 The relative concentrations (weight percentages ± SE of the lipid) of the major lipid components in the supernatant of the livers of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d v 19d	NS	*	**	***	*	NS	**	*	**	***
19d v 1DO	NS	NS	NS	NS	NS	NS	*	NS	NS	*
1DO v 6DO	NS	NS	NS	***	***	NS	*	NS	***	***
1DO v 12DO	NS	***	**	***	***	NS	*	NS	***	***

The results refer to the data shown in Table 27.
[a,b,c]- notations as in Table 15.
Significance of difference- notations as in Table 16a.

Table 28a The levels of significance for the changes in relative concentrations of the major lipid components in the supernatant of the liver during embryonic and post-hatch development.

	supernatant				
	PL[a]	FC[a]	FFA[a]	TG[a]	CE[a]
13d v 13d	NS	NS	NS	NS	NS
19d v 19d	NS	NS	NS	NS	NS
1DO v 1DO	NS	NS	NS	NS	NS
6DO v 6DO	NS	NS	NS	NS	NS
12DO v 12DO	NS	NS	***	NS	NS

The results refer to the data shown in Table 27.
[a]- notation as in Table 16b.
Significance of difference- notations as in Table 16a.

Table 28b The levels of significance for the changes in relative concentrations of the major lipid components in the supernatant of the liver between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d	27.49 ±2.27	23.18 ±3.22	10.01 ±1.08	25.82 ±3.14	13.20 ±0.10	25.32 ±2.98	24.12 ±3.01	6.89 ±2.32	21.22 ±3.22	22.45 ±2.56
16d	29.34 ±3.60	16.94 ±2.48	5.09 ±0.01	24.00 ±1.90	24.70 ±0.48	NA[d]	NA	NA	NA	NA
19d	22.70 ±1.24	18.38 ±2.70	4.91 ±0.72	21.74 ±0.80	32.26 ±2.94	19.58 ±2.74	24.99 ±4.35	6.16 ±1.70	17.36 ±0.50	31.90 ±3.81
days after hatch:										
1DO	19.60 ±3.34	16.32 ±3.82	5.10 ±1.04	25.24 ±0.04	33.70 ±0.38	24.72 ±2.28	25.02 ±2.58	6.02 ±0.88	12.73 ±0.09	31.52 ±4.66
6DO	13.94 ±3.30	14.28 ±1.52	7.40 ±3.14	49.50 ±4.76	14.96 ±3.12	18.24 ±0.28	4.53 ±2.41	3.86 ±1.22	55.44 ±2.22	12.88 ±0.34
12DO	16.98 ±3.12	14.56 ±3.08	4.36 ±0.12	55.21 ±0.97	8.88 ±2.14	18.26 ±1.42	9.20 ±1.33	4.80 ±0.46	58.18 ±3.44	9.56 ±3.06

Number of replicates as for Table 17.
[a,b,c,d]- notations as in Table 15.

Table 29 The relative concentrations (weight percentages ± SE of the lipid) of the major lipid components in the supernatant of the hearts of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d v 19d	NS	NS	**	NS	***	NS	NS	NS	NS	NS
19d v 1DO	NS	NS	NS	**	NS	NS	NS	NS	***	NS
1DO v 6DO	NS	NS	NS	**	***	*	**	NS	***	**
1DO v 12DO	NS	NS	NS	***	***	NS	**	NS	***	**

The results refer to the data shown in Table 29.
[a,b,c]- notations as in Table 15.
Significance of difference- notations as in Table 16a.

Table 30a The levels of significance for the changes in relative concentrations of the major lipid components in the supernatant of the heart during embryonic and post-hatch development.

	microsomes				
	PL[a]	FC[a]	FFA[a]	TG[a]	CE[a]
13d v 13d	NS	NS	NS	NS	*
19d v 19d	NS	NS	NS	**	NS
1DO v 1DO	NS	NS	NS	***	NS
6DO v 6DO	NS	*	NS	NS	NS
12DO v 12DO	NS	NS	NS	NS	NS

The results refer to the data shown in Table 29.
[a]- notation as in Table 16b.
Significance of difference- notations as in Table 16a.

Table 30b The levels of significance for the changes in relative concentrations of the major lipid components in the supernatant of the heart between embryos and chicks from mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d	58.51 ±2.14	27.87 ±4.17	3.13 ±1.07	5.97 ±1.40	4.48 ±1.26	61.21 ±3.26	18.42 ±3.24	2.08 ±0.63	4.21 ±0.62	14.06 ±4.21
16d	64.38 ±0.92	22.80 ±1.24	4.16 ±0.96	5.08 ±0.26	3.58 ±0.90	NA[d]	NA	NA	NA	NA
19d	58.86 ±2.50	21.92 ±4.32	8.56 ±4.94	3.03 ±0.61	7.63 ±3.28	60.30 ±6.22	15.90 ±4.60	3.30 ±1.16	3.70 ±0.28	16.81 ±4.51
days after hatch:										
1DO	56.90 ±3.04	26.90 ±0.60	2.72 ±0.32	6.56 ±1.32	6.92 ±2.21	51.81 ±6.87	21.66 ±4.22	4.22 ±0.76	7.72 ±0.98	14.68 ±3.58
6DO	39.45 ±4.99	41.65 ±3.55	4.55 ±1.16	8.82 ±2.48	5.50 ±0.10	42.63 ±6.27	34.74 ±5.50	11.72 ±2.76	4.08 ±1.20	6.84 ±0.62
12DO	30.13 ±1.32	45.24 ±5.63	6.54 ±0.73	12.41 ±3.92	5.68 ±0.60	37.08 ±6.52	40.74 ±5.82	5.47 ±2.30	12.53 ±3.01	4.18 ±1.00

Number of replicates as for Table 19.
[a,b,c,d]- notations as in Table 15.

Table 31 The relative concentrations (weight percentages ± SE of the lipid) of the major lipid components in the supernatant of the brains of embryos and chicks from mature and young parents.

	embryos and chicks from mature parents[a]					embryos and chicks from young parents[b]				
	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]	PL[c]	FC[c]	FFA[c]	TG[c]	CE[c]
13d v 19d	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
19d v 1DO	NS	NS	NS	NS	NS	NS	NS	NS	**	NS
1DO v 6DO	*	**	NS	NS	NS	NS	NS	*	NS	NS
1DO v 12DO	***	*	**	NS	NS	NS	*	NS	NS	*

The results refer to the data shown in Table 31.
[a,b,c]- notations as in Table 15.
Significance of difference- notations as in Table 16a.

Table 32a The levels of significance for the changes in relative concentrations of the major lipid components in the supernatant of the brain during embryonic and post-hatch development.

	supernatant				
	PL[a]	FC[a]	FFA[a]	TG[a]	CE[a]
13d v 13d	NS	NS	NS	NS	NS
19d v 19d	NS	NS	NS	NS	NS
1DO v 1DO	NS	NS	NS	NS	NS
6DO v 6DO	NS	NS	NS	NS	NS
12DO v 12DO	NS	NS	NS	NS	NS

The results refer to tha data shown in Table 31.
[a]- notation as in Table 16b.
Significance of difference- notations as in Table 16a.

Table 32b The levels of significance for the changes in relative concentrations of the major lipid components in the supernatant of the brain between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	27.25 ±0.62	24.02 ±0.34	19.60 ±0.14	17.52 ±0.45	22.44 ±0.27	21.32 ±0.41
palmitoleic	0.24 ±0.10	0.24 ±0.14	0.41 ±0.11	0.54 ±0.22	2.41 ±0.15	2.60 ±0.18
stearic	22.27 ±0.52	24.60 ±0.62	28.56 ±0.28	26.85 ±0.86	23.15 ±0.14	23.75 ±0.33
oleic	9.28 ±0.26	10.22 ±0.10	9.09 ±0.10	7.43 ±0.23	16.77 ±0.38	16.04 ±1.10
linoleic	8.04 ±0.38	13.76 ±0.44	15.73 ±0.32	13.55 ±0.48	16.31 ±0.17	14.97 ±0.20
linolenic	Tr [a]	Tr	Tr	0.45 ±0.29	0.44 ±0.02	0.43 ±0.03
eicosatri-enoic	0.95 ±0.08	2.06 ±0.06	1.59 ±0.26	0.74 ±0.25	1.40 ±0.47	1.82 ±0.08
arachidonic	24.98 ±0.84	20.62 ±0.62	19.37 ±0.37	19.96 ±0.87	6.80 ±0.42	5.47 ±0.34
docosapent-aenoic	Tr	Tr	Tr	0.74 ±0.09	0.72 ±0.06	0.72 ±0.06
docosahex-aenoic	6.98 ±0.24	4.48 ±0.52	5.63 ±0.20	12.21 ±0.93	9.05 ±0.49	9.67 ±0.24

The number of replicates used for analysis was- embryos 5, chicks 4.
[a]= relative concentration less than 0.1 per cent of the total fatty acids identified.

Table 33 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the liver mitochondria of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	23.42 ±0.86	NA[b]	18.03 ±0.28	18.26 ±0.44	20.75 ±0.19	19.98 ±1.04
palmitoleic	0.31 ±0.04	NA	0.90 ±0.17	0.48 ±0.03	3.51 ±0.21	3.42 ±0.55
stearic	19.45 ±0.22	NA	24.71 ±0.28	25.51 ±0.56	23.43 ±0.09	22.36 ±0.77
oleic	8.62 ±0.99	NA	7.03 ±0.10	7.94 ±0.25	18.68 ±0.55	19.91 ±1.95
linoleic	7.98 ±0.01	NA	13.49 ±0.30	12.22 ±0.25	17.43 ±0.65	17.02 ±1.27
linolenic	0.16 ±0.02	NA	0.12 ±0.01	Tr [a]	0.38 ±0.02	0.44 ±0.08
eicosatri-enoic	0.81 ±0.09	NA	0.93 ±0.09	1.32 ±0.11	1.55 ±0.06	2.12 ±0.35
arachidonic	30.01 ±0.99	NA	23.64 ±0.16	21.28 ±0.35	8.21 ±0.15	7.45 ±0.37
docosapent-aenoic	0.18 ±0.12	NA	0.14 ±0.08	0.44 ±0.15	0.55 ±0.06	0.64 ±0.22
docosahex-aenoic	8.99 ±0.47	NA	11.17 ±0.22	12.54 ±0.26	5.85 ±0.15	6.64 ±0.53

The number of replicates used for all analyses was 4.
[a]- notation as in Table 33.
[b]= no samples available due to high embryo mortality.

Table 34 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the liver mitochondria of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	***	**	***	***
	(Y)[b]	***	NS	**	NS
palmitoleic	(M)	NS	NS	***	***
	(Y)	*	NS	***	**
stearic	(M)	***	NS	**	*
	(Y)	***	NS	*	*
oleic	(M)	NS	***	***	***
	(Y)	NS	NS	***	***
linoleic	(M)	***	**	**	*
	(Y)	***	*	***	**
linolenic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	-[c]	-
eicosatri-enoic	(M)	NS	NS	NS	**
	(Y)	NS	*	NS	NS
arachidonic	(M)	***	NS	***	***
	(Y)	***	***	***	***
docosapent-aenoic	(M)	NS	***	NS	NS
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	**	***	*	*
	(Y)	**	**	***	***

The results refer to the data shown in Tables 33, 34.
[a]= results for progeny from mature parents (thirty-seven to forty weeks of age).
[b]= results for progeny from young parents (twenty-three to twenty-four weeks of age).
[c]= values too low to allow accurate comparisons.

Significance of difference: *=P<0.05 **=P<0.01 ***=P<0.001
NS= Not Significant

Table 35a The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the liver mitochondria during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	***	**	NS	**	NS
palmitoleic	NS	*	NS	**	NS
stearic	**	***	NS	NS	NS
oleic	NS	***	NS	*	NS
linoleic	NS	**	*	NS	NS
linolenic	-[a]	-	NS	NS	NS
eicosatri-enoic	NS	*	NS	NS	NS
arachidonic	**	***	NS	*	**
docosapent-aenoic	-	-	NS	NS	NS
docosahex-aenoic	**	***	NS	***	**

The results refer to the data shown in Tables 33, 34.
[a]= values too low to allow accurate comparisons.
Significance of difference- notations as in Table 35a.

Table 35b The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the liver mitochondria between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	26.99 ±1.25	26.16 ±0.54	27.79 ±1.06	21.18 ±0.64	20.89 ±1.75	17.47 ±0.27
palmitoleic	1.19 ±0.27	1.54 ±0.04	0.58 ±0.27	0.37 ±0.13	1.19 ±0.44	1.65 ±0.28
stearic	19.71 ±0.90	19.31 ±0.27	20.36 ±0.38	21.98 ±1.09	24.34 ±1.57	22.88 ±0.58
oleic	17.50 ±0.77	16.28 ±0.46	14.61 ±0.39	14.98 ±0.48	16.43 ±1.13	16.17 ±0.45
linoleic	7.35 ±0.15	11.35 ±0.08	12.22 ±0.19	13.81 ±0.18	13.48 ±2.45	27.44 ±0.80
linolenic	0.28 ±0.07	0.22 ±0.01	0.17 ±0.02	0.13 ±0.04	0.72 ±0.24	0.47 ±0.03
eicosatri-enoic	0.63 ±0.07	0.82 ±0.04	0.88 ±0.16	0.73 ±0.12	1.66 ±0.21	1.48 ±0.09
arachidonic	17.54 ±1.34	17.60 ±1.08	22.94 ±1.30	23.56 ±1.63	18.08 ±2.54	10.23 ±0.24
docosapent-aenoic	0.60 ±0.08	0.59 ±0.02	0.46 ±0.02	0.59 ±0.03	1.12 ±0.17	0.67 ±0.10
docosahex-aenoic	8.18 ±1.05	6.13 ±0.14	5.14 ±0.25	2.69 ±0.22	2.10 ±0.38	1.56 ±0.15

The number of replicates used for all analyses was 4.

Table 36 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the heart mitochondria of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	25.12 ±0.62	NA[b]	24.02 ±0.40	20.29 ±1.30	19.98 ±0.20	17.35 ±0.31
palmitoleic	1.10 ±0.54	NA	1.09 ±0.63	0.56 ±0.41	1.26 ±0.21	1.22 ±0.23
stearic	20.10 ±0.34	NA	19.70 ±1.07	19.85 ±1.07	21.49 ±0.63	28.77 ±0.15
oleic	17.51 ±0.42	NA	14.55 ±0.38	15.40 ±0.66	15.05 ±0.31	15.65 ±0.30
linoleic	8.21 ±0.12	NA	10.53 ±0.20	12.95 ±0.33	21.95 ±0.71	26.93 ±0.32
linolenic	0.21 ±0.03	NA	0.13 ±0.02	Tr [a]	0.49 ±0.14	0.45 ±0.02
eicosatri-enoic	0.76 ±0.06	NA	0.86 0.04	0.93 ±0.09	1.01 ±0.16	2.08 ±0.49
arachidonic	18.12 ±1.23	NA	23.72 ±0.44	26.12 ±0.70	16.73 ±0.49	10.94 ±0.53
docosapent-aenoic	0.24 ±0.13	NA	0.22 ±0.10	0.45 ±0.02	0.88 ±0.13	0.52 ±0.08
docosahex-aenoic	8.72 ±1.23	NA	5.18 ±0.12	3.37 ±0.05	1.17 ±0.04	0.74 ±0.05

The number of replicates used for all analyses was 4.
[a,b]- notations as in Tables 33, 34 respectively.

Table 37 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the heart mitochondria of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	**	NS	**
	(Y)[b]	NS	*	NS	NS
palmitoleic	(M)	NS	NS	NS	**
	(Y)	NS	NS	NS	NS
stearic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
oleic	(M)	*	NS	NS	NS
	(Y)	**	NS	NS	NS
linoleic	(M)	***	***	**	***
	(Y)	***	***	***	***
linolenic	(M)	NS	NS	NS	***
	(Y)	NS	-[c]	*	***
eicosatri-enoic	(M)	NS	NS	**	**
	(Y)	NS	NS	NS	NS
arachidonic	(M)	*	NS	NS	***
	(Y)	**	*	***	***
docosapent-aenoic	(M)	NS	*	*	NS
	(Y)	NS	NS	*	NS
docosahex-aenoic	(M)	*	***	NS	**
	(Y)	*	***	***	***

The results refer to the data shown in Tables 36, 37.
[a,b,c]- notations as in Table 35a.
Significance of difference- notations as in Table 35a.

Table 38a The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the heart mitochondria during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	*	NS	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	NS	NS	***
oleic	NS	NS	NS	NS	NS
linoleic	NS	***	NS	*	NS
linolenic	NS	NS	-[a]	NS	NS
eicosatri-enoic	NS	NS	NS	*	NS
arachidonic	NS	NS	NS	NS	NS
docosapent-aenoic	NS	NS	**	NS	NS
docosahex-aenoic	NS	NS	*	NS	**

The results refer to the data shown in Tables 36, 37.
[a]- notation as in Table 35b.
Significance of difference- notations as in Table 35a.

Table 38b The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the heart mitochondria between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	34.97 ±1.17	36.40 ±0.31	33.90 ±0.82	32.52 ±1.00	30.59 ±1.15	29.73 ±0.02
palmitoleic	5.72 ±0.29	3.78 ±0.26	3.59 ±0.34	2.59 ±0.42	2.47 ±0.14	2.48 ±0.32
stearic	11.28 ±1.65	13.36 ±0.30	15.73 ±0.30	17.12 ±0.30	20.16 ±0.57	19.86 ±0.81
oleic	19.10 ±0.52	16.81 ±0.32	17.08 ±0.53	16.69 ±0.32	17.95 ±0.38	18.96 ±0.45
linoleic	1.74 ±0.08	1.74 ±0.05	1.43 ±0.52	1.43 ±0.09	0.71 ±0.05	0.50 ±0.03
linolenic	0.24 ±0.02	0.23 ±0.01	0.37 ±0.04	0.51 ±0.02	0.46 ±0.09	0.63 ±0.04
eicosatri-enoic	0.37 ±0.03	0.43 ±0.05	0.56 ±0.05	0.69 ±0.07	0.32 ±0.25	0.85 ±0.22
arachidonic	8.35 ±0.46	8.11 ±0.08	8.74 ±0.26	9.58 ±0.33	10.11 ±0.44	10.05 ±0.50
docosapent-aenoic	0.70 ±0.05	0.64 ±0.01	0.55 ±0.04	0.55 ±0.02	0.40 ±0.15	0.49 ±0.05
docosahex-aenoic	17.65 ±0.47	18.51 ±0.39	17.97 ±0.37	18.33 ±0.66	16.32 ±1.12	16.45 ±0.65

The number of replicates used for all analyses was 4.

Table 39 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the brain mitochondria of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	34.86 ±1.26	NA[a]	34.92 ±0.26	32.36 ±0.34	30.01 ±0.41	28.92 ±0.22
palmitoleic	4.40 ±0.39	NA	3.89 ±0.31	4.56 ±0.13	3.75 ±0.62	4.33 ±0.56
stearic	12.62 ±1.56	NA	14.76 ±0.32	17.73 ±0.61	18.60 ±0.26	19.54 ±0.24
oleic	19.72 ±0.62	NA	16.78 ±0.18	17.01 ±0.32	19.28 ±0.56	19.34 ±0.19
linoleic	1.62 ±0.51	NA	1.57 ±0.06	1.57 ±0.07	0.96 ±0.06	0.92 ±0.10
linolenic	0.33 ±0.21	NA	0.32 ±0.01	0.49 ±0.01	0.65 ±0.03	0.60 ±0.01
eicosatri-enoic	0.35 ±0.12	NA	0.41 ±0.08	0.55 ±0.10	0.72 ±0.08	0.82 ±0.06
arachidonic	7.20 ±0.32	NA	5.63 ±0.10	9.27 ±0.14	9.79 ±0.12	9.76 ±0.31
docosapent-aenoic	0.42 ±0.02	NA	0.48 ±0.01	0.42 ±0.01	0.39 ±0.02	0.45 ±0.03
docosahex-aenoic	18.50 ±0.42	NA	18.24 ±0.32	16.89 ±0.18	15.86 ±0.28	15.32 ±0.36

The number of replicates used for all analyses was 4.
[a]= no samples available due to high embryo mortality.

Table 40 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the brain mitochondria of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	NS	*
	(Y)[b]	NS	***	**	***
palmitoleic	(M)	**	NS	NS	NS
	(Y)	NS	NS	NS	NS
stearic	(M)	*	*	**	*
	(Y)	NS	**	NS	*
oleic	(M)	*	NS	*	**
	(Y)	**	NS	*	***
linoleic	(M)	NS	NS	***	***
	(Y)	NS	NS	***	NS
linolenic	(M)	*	*	NS	*
	(Y)	NS	***	NS	***
eicosatri-enoic	(M)	*	NS	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	NS	NS	NS
	(Y)	**	***	NS	NS
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	*	**	NS	NS
docosahex-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	*	*	**

The results refer to the data shown in Tables 39, 40.
[a,b]- notations as in Table 35a.
Significance of difference- notations as in Table 35a.

Table 41a The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the brain mitochondria during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	*
palmitoleic	NS	NS	**	NS	*
stearic	NS	NS	NS	*	NS
oleic	NS	NS	NS	NS	NS
linoleic	NS	NS	NS	*	**
linolenic	NS	NS	NS	NS	NS
eicosatri-enoic	NS	NS	NS	NS	NS
arachidonic	NS	***	NS	NS	NS
docosapent-aenoic	**	NS	**	NS	NS
docosahex-aenoic	NS	NS	NS	NS	NS

The results refer to the data shown in Tables 39, 40.
Significance of difference- notations as in Table 35a.

Table 41b The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the brain mitochondria between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	27.70 ±0.33	23.43 ±0.36	20.27 ±0.45	19.60 ±0.70	24.49 ±0.29	23.83 ±0.75
palmitoleic	0.57 ±0.19	0.32 ±0.12	0.22 ±0.11	0.68 ±0.26	2.32 ±0.07	2.57 ±0.24
stearic	20.27 ±0.77	21.75 ±0.22	25.36 ±0.15	25.89 ±0.29	23.60 ±0.29	24.09 ±0.37
oleic	9.79 ±0.29	10.80 ±0.19	9.23 ±0.35	8.15 ±0.31	16.45 ±0.14	19.99 ±0.66
linoleic	7.65 ±0.18	14.55 ±0.27	14.96 ±0.32	11.45 ±0.26	15.45 ±0.22	13.89 ±0.38
linolenic	Tr [a]	Tr	Tr	0.14 ±0.04	0.47 ±0.02	0.44 ±0.05
eicosatri-enoic	0.77 ±0.14	0.70 ±0.04	0.75 ±0.04	1.13 ±0.45	2.05 ±0.23	1.81 ±0.30
arachidonic	24.57 ±0.99	21.86 ±0.25	21.01 ±0.47	18.62 ±0.45	5.25 ±0.23	3.43 ±0.30
docosapent-aenoic	0.14 ±0.02	0.15 ±0.03	0.12 ±0.01	0.88 ±0.12	0.76 ±0.03	0.58 ±0.19
docosahex-aenoic	8.54 ±0.52	6.44 ±0.35	8.10 ±0.40	13.45 ±0.84	8.79 ±0.39	9.38 ±0.11

Number of replicates as for Table 33.
[a]- notation as in Table 33.

Table 42 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the liver microsomes of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	24.85 ±0.21	NA[b]	21.34 ±0.50	20.16 ±0.21	22.97 ±0.22	22.37 ±1.40
palmitoleic	0.50 ±0.05	NA	0.72 0.16	0.40 ±0.05	3.33 ±0.08	3.26 ±0.41
stearic	22.23 ±0.04	NA	24.34 ±0.44	24.76 ±0.19	23.43 ±0.17	23.05 ±0.46
oleic	7.37 ±0.19	NA	7.99 ±0.32	8.74 ±0.24	20.04 ±0.38	20.47 ±1.85
linoleic	6.51 ±1.10	NA	12.22 ±0.51	10.56 ±0.17	16.20 ±0.55	15.62 ±1.48
linolenic	0.19 ±0.02	NA	Tr [a]	0.15 ±0.02	0.42 ±0.02	0.48 ±0.03
eicosatri-enoic	0.70 ±0.08	NA	1.80 ±0.45	1.46 ±0.17	1.65 ±0.02	2.64 ±0.33
arachidonic	27.84 ±0.18	NA	21.15 ±0.48	18.88 ±0.18	5.48 ±0.14	5.06 ±0.72
docosapent-aenoic	ND[c]	NA	Tr	0.66 ±0.02	0.59 ±0.08	0.85 ±0.10
docosahex-aenoic	9.49 ±0.01	NA	10.32 ±0.47	14.23 ±0.09	5.90 ±0.31	6.20 ±0.61

Number of replicates as for Table 34.
[a,b]- notations as for Tables 33, 34 respectively.
[c]= no detected concentration.

Table 43 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the liver microsomes of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	***	NS	***	**
	(Y)[b]	***	NS	***	NS
palmitoleic	(M)	NS	NS	***	**
	(Y)	NS	NS	***	***
stearic	(M)	***	NS	**	**
	(Y)	**	NS	**	*
oleic	(M)	NS	NS	***	***
	(Y)	NS	NS	***	***
linoleic	(M)	***	***	***	**
	(Y)	**	*	***	*
linolenic	(M)	NS	NS	***	**
	(Y)	NS	-[c]	***	***
eicosatri-enoic	(M)	NS	NS	NS	NS
	(Y)	*	NS	NS	*
arachidonic	(M)	*	**	***	***
	(Y)	***	**	***	***
docosapent-aenoic	(M)	NS	***	NS	NS
	(Y)	NS	-	NS	NS
docosahex-aenoic	(M)	NS	***	**	**
	(Y)	NS	***	***	***

The results refer to the data shown in Tables 42, 43.
[a,b,c] - notations as in Table 35a.
Significance of difference- notations as in Table 35a.

Table 44a The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the liver microsomes during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	***	NS	NS	**	NS
palmitoleic	NS	*	NS	***	NS
stearic	*	NS	*	NS	NS
oleic	***	*	NS	***	NS
linoleic	NS	**	*	NS	NS
linolenic	-[a]	NS	NS	NS	NS
eicosatri-enoic	NS	NS	NS	NS	NS
arachidonic	*	NS	NS	NS	NS
docosapent-aenoic	-	NS	NS	NS	NS
docosahex-aenoic	NS	**	NS	**	**

The results refer to the data shown in Tables 42, 43.
[a]- notation as in Table 35b.
Significance of difference- notations as in Table 35a.

Table 44b The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the liver microsomes between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	31.82 ±0.59	33.96 ±1.18	30.40 ±1.08	26.92 ±1.00	27.82 ±1.27	24.10 ±0.49
palmitoleic	1.85 ±0.27	1.73 ±0.16	1.10 ±0.15	1.27 ±0.80	1.06 ±0.87	1.86 ±0.84
stearic	16.88 ±0.60	18.45 ±0.12	19.69 ±0.58	20.62 ±0.45	18.26 ±1.00	20.27 ±0.67
oleic	17.14 ±0.31	17.49 ±0.62	16.55 ±0.45	15.81 ±0.43	17.58 ±0.28	20.07 ±0.57
linoleic	5.50 ±0.28	9.10 ±0.16	9.38 ±0.09	9.38 ±0.17	17.82 ±1.08	21.76 ±0.58
linolenic	0.21 ±0.02	0.15 ±0.03	0.18 ±0.02	0.46 ±0.20	0.38 ±0.12	0.48 ±0.08
eicosatri-enoic	0.50 ±0.17	0.85 ±0.36	0.84 ±0.02	1.02 ±0.38	1.13 ±0.18	2.15 ±0.21
arachidonic	14.56 ±0.92	12.17 ±1.27	16.70 ±1.12	20.18 ±0.94	11.23 ±0.61	5.28 ±0.57
docosapent-aenoic	0.93 ±0.09	0.52 ±0.04	0.48 ±0.05	0.76 ±0.02	1.35 ±0.15	1.11 ±0.09
docosahex-aenoic	10.32 ±1.00	5.59 ±0.39	4.67 ±0.93	3.83 ±0.21	3.36 ±0.12	2.90 ±0.16

Number of replicates as for Table 36.

Table 45 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the heart microsomes of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	31.45 ±0.89	NA[a]	31.33 ±0.68	28.83 ±0.82	25.83 ±0.63	24.71 ±1.80
palmitoleic	0.81 ±0.24	NA	0.59 ±0.34	1.10 ±0.38	2.59 ±0.53	1.75 ±0.31
stearic	16.24 ±0.52	NA	17.50 ±0.42	17.93 ±0.55	19.51 ±0.25	21.15 ±0.36
oleic	18.12 ±1.24	NA	16.54 ±0.43	15.54 ±0.13	17.66 ±0.36	17.82 ±0.56
linoleic	6.26 ±0.12	NA	7.76 ±0.16	7.93 ±0.40	17.79 ±0.58	20.92 ±2.30
linolenic	0.25 ±0.04	NA	0.14 ±0.03	0.08 ±0.04	0.52 ±0.02	0.71 ±0.07
eicosatri-enoic	1.24 ±0.32	NA	1.30 ±0.23	1.49 ±0.20	2.07 ±0.06	3.33 ±0.39
arachidonic	15.42 ±0.62	NA	18.67 ±0.94	22.12 ±0.50	11.17 ±0.49	6.50 ±0.59
docosapent-aenoic	0.35 ±0.06	NA	0.36 ±0.04	0.60 ±0.09	0.89 ±0.32	1.15 ±0.08
docosahex-aenoic	9.86 ±1.20	NA	5.87 ±0.52	4.35 ±0.18	1.67 ±0.24	1.97 ±0.14

Number of replicates as for Table 37.
[a]= no samples available due to high embryo mortality.

Table 46 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the heart microsomes of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	NS	*
	(Y)[b]	NS	NS	*	*
palmitoleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
stearic	(M)	*	NS	NS	NS
	(Y)	NS	NS	*	**
oleic	(M)	NS	NS	*	***
	(Y)	NS	NS	**	**
linoleic	(M)	***	NS	***	***
	(Y)	***	NS	***	**
linolenic	(M)	NS	NS	NS	*
	(Y)	NS	NS	***	***
eicosatri-enoic	(M)	NS	NS	*	NS
	(Y)	NS	NS	*	**
arachidonic	(M)	NS	NS	***	***
	(Y)	*	*	***	***
docosapent-aenoic	(M)	NS	NS	***	**
	(Y)	NS	*	NS	**
docosahex-aenoic	(M)	**	NS	NS	*
	(Y)	*	*	***	***

The results refer to the data shown in Tables 45, 46.
[a,b]- notations as in Table 35a.
Significance of difference- notations as in Table 35a.

Table 47a The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the heart microsomes during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	NS
palmitoleic	*	NS	NS	NS	NS
stearic	NS	*	**	NS	NS
oleic	NS	NS	NS	NS	*
linoleic	*	***	*	NS	NS
linolenic	NS	NS	NS	NS	NS
eicosatri-enoic	NS	NS	NS	**	*
arachidonic	NS	NS	NS	NS	NS
docosapent-aenoic	**	NS	NS	NS	NS
docosahex-aenoic	NS	NS	NS	***	**

The results refer to the data shown in Tables 45, 46.
Significance of difference- notations as in Table 35a.

Table 47b The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the heart microsomes between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	35.60 ±0.25	36.44 ±1.67	36.07 ±0.77	34.50 ±0.26	34.06 ±1.00	31.45 ±0.24
palmitoleic	5.14 ±0.07	3.80 ±0.36	2.17 ±0.61	3.21 ±0.48	3.53 ±0.41	3.04 ±0.41
stearic	12.77 ±0.21	13.49 ±0.27	15.80 ±0.36	16.78 ±0.68	19.45 ±0.85	18.96 ±0.52
oleic	17.24 ±0.22	16.92 ±0.09	16.72 ±0.55	16.05 ±0.06	18.04 ±0.28	19.29 ±0.45
linoleic	1.00 ±0.33	1.50 ±0.06	1.61 ±0.15	1.23 ±0.04	0.90 ±0.12	0.54 ±0.04
linolenic	0.22 ±0.01	0.19 ±0.04	0.30 ±0.01	0.40 ±0.01	0.49 ±0.03	0.52 ±0.09
eicosatri-enoic	0.33 ±0.01	0.33 ±0.13	0.56 ±0.05	0.62 ±0.05	0.51 ±0.03	0.68 ±0.06
arachidonic	7.27 ±0.15	7.51 ±0.22	8.03 ±0.23	9.11 ±0.12	8.88 ±0.40	9.05 ±0.08
docosapent-aenoic	0.77 ±0.02	0.50 ±0.17	0.68 ±0.06	0.54 ±0.02	0.41 ±0.06	0.59 ±0.06
docosahex-aenoic	19.69 ±0.27	19.11 ±1.14	18.06 ±0.19	17.55 ±0.07	13.74 ±0.98	15.88 ±0.19

Number of replicates as for Table 39.

Table 48 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the brain microsomes of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	35.12 ±0.52	NA[b]	36.47 ±0.45	34.00 ±0.55	32.46 ±0.81	30.63 ±0.25
palmitoleic	5.46 ±0.56	NA	4.03 ±0.09	2.58 ±0.61	3.79 ±0.77	4.40 ±0.50
stearic	13.10 ±0.40	NA	15.73 ±0.30	18.33 ±0.19	19.59 ±0.42	19.49 ±0.24
oleic	16.12 ±0.62	NA	15.58 ±0.06	15.69 ±0.29	17.40 ±0.19	18.97 ±0.06
linoleic	1.52 ±0.07	NA	1.32 ±0.05	1.13 ±0.04	0.74 ±0.16	0.78 ±0.07
linolenic	0.22 ±0.02	NA	0.21 ±0.01	0.39 ±0.01	0.49 ±0.04	0.52 ±0.02
eicosatri-enoic	0.42 ±0.03	NA	0.44 ±0.09	0.43 ±0.13	0.58 ±0.08	0.65 ±0.10
arachidonic	7.12 ±0.14	NA	7.65 ±0.16	9.27 ±0.14	9.26 ±0.14	9.59 ±0.09
docosapent-aenoic	0.72 ±0.18	NA	0.48 ±0.02	0.25 ±0.12	0.30 ±0.11	Tr [a]
docosahex-aenoic	20.20 ±0.92	NA	18.10 ±0.35	17.94 ±0.21	15.40 ±0.21	14.86 ±0.19

Number of replicates as for Table 40.
[a,b]- notations as in Tables 33, 34 respectively.

Table 49 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the brain microsomes of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	NS	***
	(Y)[b]	NS	*	NS	**
palmitoleic	(M)	**	NS	NS	NS
	(Y)	*	NS	NS	NS
stearic	(M)	***	NS	*	*
	(Y)	**	***	*	**
oleic	(M)	NS	NS	***	***
	(Y)	NS	NS	**	***
linoleic	(M)	NS	*	*	***
	(Y)	NS	*	NS	**
linolenic	(M)	**	***	*	NS
	(Y)	NS	***	NS	***
eicosatri-enoic	(M)	**	NS	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	*	**	NS	NS
	(Y)	NS	***	NS	NS
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	-[c]
docosahex-aenoic	(M)	**	*	**	***
	(Y)	NS	NS	***	***

The results refer to the data shown in Tables 48, 49.
[a,b,c]- notations as in Table 35a.
Significance of difference- notations as in Table 35a.

Table 50a The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the brain microsomes during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	NS
palmitoleic	NS	*	NS	NS	NS
stearic	NS	NS	NS	NS	NS
oleic	NS	NS	NS	NS	NS
linoleic	NS	NS	NS	NS	*
linolenic	NS	***	NS	NS	NS
eicosatri-enoic	NS	NS	NS	NS	NS
arachidonic	NS	NS	NS	NS	**
docosapent-aenoic	NS	*	NS	NS	***
docosahex-aenoic	NS	NS	NS	NS	**

The results refer to the data shown in Tables 48, 49.
Significance of difference- notations as in Table 35a.

Table 50b The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the brain microsomes between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	29.59 ±1.79	23.54 ±1.44	22.74 ±1.68	20.01 ±0.17	25.43 ±1.52	26.48 ±1.87
palmitoleic	ND[b]	ND	0.19 ±0.14	0.21 ±0.14	2.17 ±0.10	0.82 ±0.26
stearic	17.95 ±0.80	18.68 ±1.04	21.54 ±0.85	23.60 ±1.35	23.84 ±0.40	22.79 ±2.79
oleic	14.98 ±1.72	14.94 ±0.50	12.75 ±0.67	10.71 ±0.58	18.79 ±0.02	20.67 ±0.15
linoleic	8.52 ±0.92	15.00 ±0.55	15.71 ±0.47	13.42 ±0.43	16.49 ±0.45	15.88 ±0.24
linolenic	Tr [a]	ND	Tr	0.33 ±0.13	0.24 ±0.14	Tr
eicosatri-enoic	0.30 ±0.11	0.33 ±0.21	0.68 ±0.14	0.42 ±0.02	1.59 ±0.68	6.13 ±0.24
arachidonic	21.00 ±1.65	21.83 ±2.28	19.96 ±0.96	18.49 ±0.74	4.98 ±0.68	2.59 ±0.99
docosapent-aenoic	0.19 ±0.03	0.16 ±0.10	0.14 ±0.06	0.44 ±0.24	ND	ND
docosahex-aenoic	7.47 ±0.73	5.51 ±1.30	6.27 ±1.41	12.37 ±0.03	6.48 ±0.31	4.79 ±0.62

Number of replicates as for Table 33.
[a]- notation as in Table 33.
[b]= no detected concentration.

Table 51 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the liver supernatant of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	26.50 ±2.12	NA[b]	18.58 ±2.81	21.18 ±0.96	23.51 ±0.65	21.48 ±1.49
palmitoleic	0.21 ±0.12	NA	1.25 ±1.07	0.53 ±0.23	3.56 ±0.13	2.76 ±0.95
stearic	18.55 ±0.77	NA	23.48 ±0.66	23.06 ±0.35	21.78 ±1.48	22.88 ±0.92
oleic	11.82 ±1.34	NA	16.44 ±4.78	11.17 ±0.31	22.64 ±1.86	21.01 ±4.28
linoleic	7.88 ±1.24	NA	16.33 ±1.05	12.74 ±0.98	16.52 ±1.24	16.38 ±2.32
linolenic	Tr [a]	NA	Tr	Tr	0.14 ±0.10	0.48 ±0.12
eicosatri-enoic	0.59 ±0.04	NA	0.58 ±0.02	1.29 ±0.65	1.71 ±0.13	2.58 ±0.67
arachidonic	26.27 ±2.27	NA	15.98 ±2.99	18.95 ±0.28	5.00 ±0.58	5.43 ±1.19
docosapent-aenoic	0.11 ±0.12	NA	0.17 ±0.07	0.88 ±0.41	Tr	0.74 ±0.26
docosahex-aenoic	8.00 ±0.14	NA	7.20 ±0.11	9.74 ±2.37	5.07 ±0.69	6.28 ±1.24

Number of replicates as for Table 34.
[a,b]- notations as in Tables 33, 34 respectively.

Table 52 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the liver supernatant of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	*	NS	*	*
	(Y)[b]	NS	NS	NS	NS
palmitoleic	(M)	NS	NS	***	NS
	(Y)	NS	NS	***	NS
stearic	(M)	NS	NS	NS	NS
	(Y)	**	NS	NS	NS
oleic	(M)	NS	NS	***	***
	(Y)	NS	NS	***	NS
linoleic	(M)	***	**	**	**
	(Y)	**	*	NS	NS
linolenic	(M)	NS	NS	NS	-[c]
	(Y)	NS	NS	NS	NS
eicosatri-enoic	(M)	NS	NS	NS	***
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	NS	***	***
	(Y)	*	NS	***	***
docosapent-aenoic	(M)	NS	NS	-	-
	(Y)	NS	NS	-	NS
docosahex-aenoic	(M)	NS	**	***	***
	(Y)	**	NS	NS	NS

The results refer to the data shown in Tables 51, 52.
[a,b,c]- notations as in Table 35a.
Significance of differences- notations as in Table 35a.

Table 53a The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the liver supernatant during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	NS
palmitoleic	-[a]	NS	NS	***	NS
stearic	NS	NS	NS	NS	NS
oleic	NS	NS	NS	NS	NS
linoleic	NS	NS	NS	NS	NS
linolenic	NS	NS	-	NS	-
eicosatri-enoic	*	NS	NS	NS	**
arachidonic	NS	NS	NS	NS	NS
docosapent-aenoic	NS	NS	NS	NS	-
docosahex-aenoic	NS	NS	NS	NS	NS

The results refer to the data shown in Tables 51, 52.
[a]- notation as in Table 35b.
Significance of difference- notations as in Table 35a.

Table 53b The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the liver supernatant between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	30.48 ±1.40	36.18 ±4.76	27.80 ±0.06	29.64 ±1.56	26.83 ±0.33	25.05 ±2.99
palmitoleic	0.72 ±0.28	1.14 ±0.60	1.14 ±0.84	0.45 ±0.15	1.07 ±0.66	1.01 ±0.11
stearic	18.41 ±1.12	16.84 ±1.28	20.91 ±1.48	18.18 ±3.38	19.09 ±0.84	17.80 ±2.15
oleic	21.48 ±1.93	19.97 ±1.01	17.59 ±1.41	18.54 ±1.70	21.09 ±0.06	23.01 ±1.27
linoleic	10.86 ±1.29	13.75 ±3.32	15.18 ±0.46	15.07 ±0.43	18.50 ±1.54	23.23 ±1.71
linolenic	0.46 ±0.15	0.38 ±0.32	Tr [a]	0.12 ±0.02	0.47 ±0.09	0.34 ±0.14
eicosatri-enoic	0.51 ±0.16	0.40 ±0.29	0.12 ±0.04	0.99 ±0.54	2.56 ±1.03	1.06 ±0.74
arachidonic	11.81 ±2.04	9.40 ±2.81	14.33 ±2.74	14.49 ±0.08	6.58 ±1.28	4.27 ±0.18
docosapent-aenoic	0.22 ±0.11	0.18 ±0.09	0.15 ±0.12	Tr	1.03 ±0.13	0.81 ±0.23
docosahex-aenoic	5.40 ±1.35	1.74 ±0.89	2.71 ±0.06	2.44 ±0.43	2.96 ±0.02	3.36 ±0.37

Number of replicates as for Table 36.
[a]- notation as in Table 33

Table 54 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the heart supernatant of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	31.52 ±2.14	NA[b]	32.47 ±2.19	33.40 ±2.30	25.31 ±1.13	23.43 ±0.55
palmitoleic	0.72 ±0.12	NA	0.80 ±0.20	1.04 ±0.06	2.47 ±0.09	2.54 ±1.08
stearic	21.52 ±1.62	NA	22.84 ±1.46	22.58 ±2.18	21.72 ±0.44	22.52 ±0.99
oleic	22.42 ±1.62	NA	17.40 ±0.93	20.45 ±1.81	19.03 ±2.30	20.00 ±1.67
linoleic	10.86 ±1.01	NA	11.09 ±1.18	9.22 ±1.58	21.21 ±1.36	23.82 ±2.03
linolenic	0.22 ±0.11	NA	0.14 ±0.04	0.13 ±0.06	0.38 ±0.04	1.28 ±0.93
eicosatri-enoic	Tr [a]	NA	0.22 ±0.02	0.32 ±0.12	0.22 ±0.12	0.35 ±0.15
arachidonic	11.49 ±1.24	NA	14.70 ±2.54	12.31 ±3.33	7.97 ±0.05	4.44 ±0.21
docosapent-aenoic	Tr	NA	ND[c]	Tr	Tr	Tr
docosahex-aenoic	1.23 ±0.14	NA	0.34 ±0.14	0.56 ±0.36	1.04 ±0.14	0.92 ±0.62

Number of replicates as for Table 37.
[a,b]- notations as in Tables 33, 34 respectively.
[c]= no detected concentration.

Table 55 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the heart supernatant of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	NS	NS
	(Y)[b]	NS	NS	*	**
palmitoleic	(M)	NS	NS	NS	*
	(Y)	NS	NS	***	NS
stearic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	NS	NS	NS
	(Y)	*	NS	NS	NS
linoleic	(M)	*	NS	NS	**
	(Y)	NS	NS	**	**
linolenic	(M)	NS	-[c]	NS	NS
	(Y)	NS	NS	*	NS
eicosatri-enoic	(M)	NS	NS	NS	NS
	(Y)	-	NS	NS	NS
arachidonic	(M)	NS	NS	***	***
	(Y)	NS	NS	NS	NS
docosapent-aenoic	(M)	NS	-	***	*
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	NS	NS	NS	NS
	(Y)	**	NS	NS	NS

The results refer to the data shown in Tables 54, 55.
[a,b,c]- notations as in Table 35a.
Significance of difference- notations as in Table 35a.

Table 56a The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the heart supernatant during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	NS
palmitoleic	NS	NS	*	NS	NS
stearic	NS	NS	NS	*	NS
oleic	NS	NS	NS	NS	NS
linoleic	NS	*	*	NS	NS
linolenic	NS	-[a]	NS	NS	NS
eicosatri-enoic	-	NS	NS	NS	NS
arachidonic	NS	NS	NS	NS	NS
docosapent-aenoic	-	NS	NS	**	NS
docosahex-aenoic	*	***	*	***	*

The results refer to the data shown in Tables 54, 55.
[a]- notation as in Table 35b.
Significance of difference- notations as in Table 35a.

Table 56b The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the heart supernatant between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	41.73 ±0.85	38.29 ±0.82	34.71 ±0.19	34.56 ±0.52	32.78 ±0.15	33.62 ±0.82
palmitoleic	3.40 ±1.73	3.95 ±0.51	2.85 ±0.49	2.81 ±0.22	1.63 ±0.63	1.33 ±0.36
stearic	11.77 ±2.31	12.84 ±0.62	14.74 ±0.47	17.05 ±1.01	17.21 ±0.72	20.46 ±2.28
oleic	15.72 ±1.82	17.26 ±0.60	17.09 ±0.13	17.07 ±0.02	21.01 ±2.09	22.55 ±0.05
linoleic	0.82 ±0.48	1.87 ±0.01	2.23 ±0.75	2.79 ±0.32	2.50 ±1.07	1.06 ±0.01
linolenic	0.16 ±0.03	0.19 ±0.01	0.33 ±0.01	0.76 ±0.30	0.57 ±0.12	0.35 ±0.15
eicosatri-enoic	0.14 ±0.10	0.43 ±0.21	0.52 ±0.13	0.23 ±0.13	0.20 ±0.14	1.26 ±0.52
arachidonic	6.63 ±1.31	7.55 ±0.08	8.72 ±0.61	8.88 ±0.02	9.31 ±0.38	7.49 ±1.39
docosapent-aenoic	0.56 ±0.15	0.42 ±0.10	1.76 ±1.14	0.44 ±0.14	0.47 ±0.05	Tr [a]
docosahex-aenoic	19.08 ±1.51	17.20 ±1.30	17.03 ±0.68	14.75 ±0.06	14.33 ±0.46	12.10 ±0.80

Number of replicates as for Table 39.
[a]- notation as in Table 33.

Table 57 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the brain supernatant of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	42.23 ±2.36	NA [a]	41.83 ±3.11	40.34 ±3.92	33.92 ±4.27	34.23 ±3.84
palmitoleic	3.79 ±0.29	NA	3.91 ±0.02	2.43 ±0.84	2.74 ±0.18	3.15 ±1.12
stearic	11.00 ±2.11	NA	14.23 ±1.26	18.65 ±0.48	18.70 ±1.18	17.73 ±0.89
oleic	16.72 ±1.12	NA	18.29 ±0.11	18.97 ±0.17	20.86 ±1.44	22.69 ±0.91
linoleic	0.92 ±0.30	NA	1.35 ±0.42	1.45 ±0.30	1.06 ±0.33	1.16 ±0.51
linolenic	0.20 ±0.12	NA	0.21 ±0.06	0.40 ±0.02	0.32 ±0.12	0.63 ±0.04
eicosatri-enoic	0.21 ±0.11	NA	0.38 ±0.02	0.57 ±0.06	1.90 ±0.13	0.81 ±0.37
arachidonic	7.12 ±0.51	NA	7.87 ±0.28	7.97 ±0.30	8.15 ±0.95	8.49 ±0.01
docosapent-aenoic	0.72 ±0.17	NA	0.15 ±0.06	0.18 ±0.02	0.35 ±0.15	0.32 ±0.01
docosahex-aenoic	16.24 ±0.72	NA	14.02 ±0.52	11.54 ±0.68	12.00 ±1.74	10.78 0.83

Number of replicates as for Table 40.
[a]= no samples available due to high embryo mortality.

Table 58 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the phospholipids of the brain supernatant of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	**	NS	*	NS
	(Y)[b]	NS	NS	NS	NS
palmitoleic	(M)	NS	NS	NS	*
	(Y)	NS	NS	NS	NS
stearic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	*	NS
oleic	(M)	NS	NS	NS	***
	(Y)	NS	NS	NS	**
linoleic	(M)	NS	NS	NS	**
	(Y)	NS	*	NS	NS
linolenic	(M)	**	NS	NS	NS
	(Y)	NS	*	NS	**
eicosatri-enoic	(M)	NS	NS	NS	NS
	(Y)	NS	*	***	NS
arachidonic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
docosapent-aenoic	(M)	NS	NS	NS	***
	(Y)	*	NS	NS	***
docosahex-aenoic	(M)	NS	*	NS	*
	(Y)	*	*	NS	NS

The results refer to the data shown in Tables 57, 58.
[a,b] - notations as in Table 35a.
Significance of difference- notations as in Table 35a.

Table 59a The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the brain supernatant during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	NS	NS	NS
oleic	NS	***	***	NS	NS
linoleic	NS	NS	*	NS	NS
linolenic	NS	NS	NS	NS	NS
eicosatri-enoic	NS	NS	NS	***	NS
arachidonic	NS	NS	*	NS	NS
docosapent-aenoic	NS	NS	NS	NS	***
docosahex-aenoic	NS	*	**	NS	NS

The results refer to the data shown in Tables 57, 58.
Significance of difference- notations as for Table 35a.

Table 59b The levels of significance for the changes in relative concentrations of the major fatty acid components in the phospholipids of the brain supernatant between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	39.97 ±0.96	32.07 ±1.40	46.79 ±3.20	33.56 ±1.79	34.46 ±0.96	33.66 ±1.59
palmitoleic	Tr [a]	1.80 ±1.11	1.56 ±0.23	2.18 ±0.77	4.84 ±0.24	5.18 ±0.38
stearic	8.77 ±0.98	8.69 ±0.64	9.36 ±1.15	10.60 ±0.98	14.06 ±0.92	13.80 ±0.91
oleic	29.63 ±1.58	30.64 ±1.03	25.78 ±0.90	34.81 ±0.63	36.37 ±0.74	41.47 ±1.27
linoleic	5.67 ±0.84	8.55 ±0.53	6.25 ±0.78	13.13 ±1.73	6.97 ±0.34	4.81 ±0.55
linolenic	1.14 ±0.64	0.28 ±0.07	0.38 ±0.22	ND[b]	0.78 ±0.34	0.51 ±0.30
eicosatri-enoic	Tr	Tr	Tr	ND	ND	ND
arachidonic	3.32 ±0.67	3.15 ±0.28	1.34 ±0.43	2.86 ±0.34	0.33 ±0.05	Tr
docosapent-aenoic	ND	0.52 ±0.06	0.22 ±0.14	0.40 ±0.24	0.30 ±0.06	Tr
docosahex-aenoic	11.59 ±2.25	14.30 ±0.81	8.34 ±2.67	2.46 ±0.86	0.71 ±0.17	0.30 ±0.10

The number of replicates used for analyses was-embryos 5, chicks 4.
[a]= relative concentration less than 0.1 per cent of the total fatty acids identified.
[b]= no detected concentration.

Table 60 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the liver mitochondria of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	29.20 ±0.16	NA[c]	38.40 ±1.14	31.14 ±0.65	32.28 ±0.65	28.63 ±3.86
palmitoleic	3.96 ±1.22	NA	1.16 ±0.63	1.26 ±0.44	7.52 ±0.76	6.13 ±0.54
stearic	9.24 ±0.80	NA	11.79 ±2.15	10.53 ±0.49	9.72 ±0.79	8.42 ±0.79
oleic	27.89 ±1.03	NA	25.00 ±1.69	33.82 ±0.48	41.86 ±0.68	45.66 ±1.82
linoleic	6.87 ±0.09	NA	5.49 ±1.49	14.23 ±0.50	7.22 ±0.16	8.49 ±2.26
linolenic	0.56 ±0.14	NA	0.99 ±0.46	0.64 ±0.04	0.99 ±0.06	1.18 ±0.39
eicosatri-enoic	0.19 ±0.02	NA	ND[b]	ND	ND	0.27 ±0.22
arachidonic	4.49 ±0.02	NA	4.27 ±0.79	3.20 ±0.19	0.26 ±0.02	0.21 ±0.09
docosapent-aenoic	0.61 ±0.08	NA	0.38 ±0.13	ND	Tr [a]	0.49 ±0.16
docosahex-aenoic	16.99 ±1.00	NA	12.72 ±4.33	5.19 ±0.42	0.27 ±0.16	0.50 ±0.18

The number of replicates used for all analyses was 4.
[a,b]- notations as in Table 60.
[c]= no samples available due to high embryo mortality.

Table 61 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the liver mitochondria of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	**	NS	NS
	(Y)[b]	***	**	NS	NS
palmitoleic	(M)	***	NS	*	*
	(Y)	NS	NS	***	***
stearic	(M)	NS	NS	*	NS
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	***	NS	**
	(Y)	NS	**	***	***
linoleic	(M)	NS	**	*	**
	(Y)	NS	**	***	NS
linolenic	(M)	NS	-[c]	-	-
	(Y)	NS	NS	**	NS
eicosatri-enoic	(M)	NS	NS	NS	NS
	(Y)	-	NS	NS	NS
arachidonic	(M)	*	*	***	***
	(Y)	NS	NS	***	***
docosapent-aenoic	(M)	-	NS	NS	-
	(Y)	NS	-	NS	-
docosahex-aenoic	(M)	NS	NS	NS	*
	(Y)	NS	NS	***	***

The results refer to the data shown in Tables 60, 61.
[a]= results for progeny from mature parents (thirty-seven to forty weeks of age).
[b]= results for progeny from young parents (twenty-three to twenty-four weeks of age).
[c]= values too low to allow accurate comparisons.
Significance of difference: *=P<0.05 **=P<0.01 ***=P<0.001
NS= Not Significant

Table 62a The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the liver mitochondria during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	***	*	NS	NS	NS
palmitoleic	*	NS	NS	*	NS
stearic	NS	NS	NS	*	**
oleic	NS	NS	NS	**	NS
linoleic	NS	NS	NS	NS	NS
linolenic	NS	NS	***	NS	NS
eicosatri-enoic	-[a]	NS	NS	NS	-
arachidonic	NS	*	NS	NS	NS
docosapent-aenoic	***	NS	NS	-	-
docosahex-aenoic	NS	NS	*	NS	NS

The results refer to the data shown in Tables 60, 61.
[a]= values too low to allow accurate comparisons.
Significance of difference- notations as in Table 62a.

Table 62b The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides on the liver mitochondria between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	27.39 ±0.96	32.93 ±1.13	31.91 ±1.04	29.74 ±2.20	27.45 ±0.83	26.59 ±0.57
palmitoleic	6.10 ±0.20	8.18 ±2.23	5.98 ±0.56	2.43 ±0.98	6.99 ±0.32	6.90 ±0.31
stearic	9.10 ±0.62	13.03 ±0.48	10.40 ±0.84	7.75 ±0.78	7.48 ±0.42	7.38 ±0.36
oleic	38.94 ±1.59	28.34 ±1.83	34.87 ±1.08	42.79 ±0.88	38.57 ±1.19	41.89 ±0.72
linoleic	9.39 ±2.30	8.85 ±0.64	12.47 ±0.77	15.40 ±0.46	16.05 ±1.26	14.57 ±0.68
linolenic	1.24 ±0.25	0.86 ±0.08	0.58 ±0.20	0.56 ±0.14	1.76 ±0.16	1.33 ±0.37
eicosatri-enoic	0.16 ±0.09	0.30 ±0.15	0.57 ±0.38	Tr [a]	1.11 ±0.38	0.55 ±0.12
arachidonic	2.04 ±0.36	1.81 ±0.24	1.94 ±0.29	0.77 ±0.12	0.11 ±0.05	Tr
docosapent-aenoic	0.84 ±0.41	1.09 ±0.23	0.37 ±0.14	0.29 ±0.18	0.25 ±0.10	0.13 ±0.11
docosahex-aenoic	4.80 0.44	4.61 ±0.76	1.00 ±0.53	0.25 ±0.14	0.23 ±0.09	Tr

The number of replicates used for all analyses was 4.
[a]- notation as in Table 60.

Table 63 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the heart mitochondria of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	29.32 ±1.22	NA[c]	34.61 ±3.60	27.19 ±2.70	26.13 ±0.86	25.83 ±0.71
palmitoleic	6.12 ±0.63	NA	5.62 ±1.52	8.51 ±0.72	7.26 ±0.29	6.93 ±0.38
stearic	11.12 ±0.23	NA	13.13 ±0.58	9.28 ±2.80	6.29 ±0.53	7.17 ±0.13
oleic	36.21 ±1.21	NA	31.01 ±1.00	44.65 ±1.57	39.72 ±0.42	41.85 ±0.25
linoleic	9.12 ±1.24	NA	8.54 ±2.13	12.75 ±1.34	17.85 ±1.22	15.27 ±0.85
linolenic	0.62 ±0.24	NA	0.54 ±0.33	0.59 ±0.46	1.83 ±0.22	2.01 ±0.15
eicosatri-enoic	Tr[a]	NA	ND[b]	ND	Tr	Tr
arachidonic	2.62 ±0.52	NA	2.96 ±0.48	1.09 ±0.53	1.00 ±0.30	0.76 ±0.69
docosapent-aenoic	0.38 ±0.21	NA	0.44 ±0.28	Tr	0.21 ±0.16	Tr
docosahex-aenoic	4.48 ±0.46	NA	3.15 ±1.46	0.79 ±0.56	0.22 ±0.03	Tr

The number of replicates used for all analyses was 4.
[a,b,c]- notations as in Tables 60, 61 respectively.

Table 64 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the heart mitochondria of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	*	NS	NS	NS
	(Y)[b]	NS	NS	NS	NS
palmitoleic	(M)	NS	*	**	**
	(Y)	NS	NS	**	**
stearic	(M)	NS	NS	NS	NS
	(Y)	*	NS	NS	NS
oleic	(M)	NS	**	*	NS
	(Y)	*	***	*	NS
linoleic	(M)	NS	*	NS	NS
	(Y)	NS	NS	*	NS
linolenic	(M)	NS	NS	**	NS
	(Y)	NS	NS	NS	*
eicosatri-enoic	(M)	NS	-[c]	*	**
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	**	**	***
	(Y)	NS	*	NS	NS
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	-	-	NS
docosahex-aenoic	(M)	**	NS	NS	-
	(Y)	NS	NS	NS	-

The results refer to the data shown in Tables 63, 64.
[a,b,c]- notations as in Table 62a.
Significance of difference- notations as in Table 62a.

Table 65a The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the heart mitochondria during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	*	*	NS	NS	NS
oleic	NS	NS	NS	NS	NS
linoleic	NS	NS	NS	NS	NS
linolenic	NS	NS	NS	NS	NS
eicosatri-enoic	NS	-[a]	NS	*	**
arachidonic	NS	NS	NS	*	NS
docosapent-aenoic	NS	NS	-	NS	NS
docosahex-aenoic	NS	NS	NS	NS	NS

The results refer to the data shown in Tables 63, 64.
[a]- notation as in Table 62b.
Significance of difference- notations as in Table 62a.

Table 65b The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the heart mitochondria between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	43.23 ±0.37	41.39 ±3.05	36.54 ±3.11	32.58 ±2.13	36.89 ±2.59	36.96 ±1.10
palmitoleic	7.79 ±0.57	7.60 ±0.90	2.98 ±0.18	3.62 ±0.43	5.71 ±0.55	5.08 ±0.36
stearic	9.20 ±1.00	12.28 ±0.46	11.23 ±1.67	9.43 ±0.93	11.33 ±1.69	15.74 ±0.23
oleic	25.66 ±0.83	25.46 ±2.45	25.69 ±1.34	38.22 ±2.30	32.97 ±2.17	34.47 ±0.51
linoleic	3.80 ±0.14	4.26 ±0.77	13.86 ±2.94	11.97 ±1.16	9.60 ±2.42	6.02 ±0.24
linolenic	0.61 ±0.21	0.40 ±0.23	2.21 ±1.21	0.95 ±0.03	0.88 ±0.33	0.40 ±0.25
eicosatri-enoic	0.38 ±0.23	0.22 ±0.14	ND[b]	0.22 ±0.10	ND	Tr [a]
arachidonic	2.76 ±0.24	2.84 ±0.42	3.99 ±0.88	1.65 ±0.35	1.41 ±0.51	0.68 ±0.40
docosapent-aenoic	Tr	Tr	ND	0.19 ±0.06	0.18 ±0.15	Tr
docosahex-aenoic	6.55 ±0.84	5.54 ±0.93	3.47 ±0.39	1.17 ±0.03	1.04 ±0.29	0.49 ±0.43

The number of replicates for all analyses was 4.
[a,b]- notations as in Table 60.

Table 66 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the brain mitochondria of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	44.24 ±1.24	NA[c]	46.02 ±1.26	45.08 ±1.82	37.69 ±0.68	33.87 ±2.95
palmitoleic	5.12 ±0.36	NA	3.04 ±0.26	3.79 ±0.15	6.50 ±0.55	6.64 ±0.60
stearic	12.12 ±0.62	NA	13.01 ±1.06	15.13 ±1.19	13.74 ±1.21	10.90 ±2.53
oleic	25.71 ±1.33	NA	25.69 ±1.44	26.63 ±1.23	34.13 ±0.36	36.36 ±1.75
linoleic	3.71 ±0.52	NA	3.80 ±0.48	4.46 ±0.22	7.94 ±0.76	10.76 ±2.66
linolenic	0.42 ±0.30	NA	0.92 ±0.08	0.61 ±0.31	Tr [a]	0.91 ±0.53
eicosatri-enoic	Tr	NA	Tr	ND[b]	ND	ND
arachidonic	3.72 ±0.32	NA	3.66 ±0.22	4.30 ±0.72	Tr	0.55 ±0.32
docosapent-aenoic	ND	ND	ND	ND	ND	ND
docosahex-aenoic	4.96 ±1.32	NA	3.85 ±1.31	ND	ND	ND

The number of replicates used for all analyses was 4.
[a,b,c]- notations as in Tables 60, 61 respectively.

Table 67 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the brain mitochondria of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	NS	NS
	(Y)[b]	NS	NS	*	*
palmitoleic	(M)	***	NS	*	*
	(Y)	**	NS	NS	NS
stearic	(M)	NS	NS	NS	***
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	**	NS	NS
	(Y)	NS	NS	**	**
linoleic	(M)	*	NS	NS	**
	(Y)	NS	NS	**	NS
linolenic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	-[c]	NS
eicosatri-enoic	(M)	-	-	-	-
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	***	**
docosapent-aenoic	(M)	NS	-	NS	-
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	*	**	NS	NS
	(Y)	NS	*	NS	NS

The results refer to the data shown in Tables 66, 67.
[a,b,c]- notations as in Table 62a.
Significance of difference- notations as in Table 62a.

Table 68a The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the brain mitochondria during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	*	**	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	**	NS	NS
oleic	NS	NS	**	NS	NS
linoleic	NS	*	***	NS	NS
linolenic	NS	NS	NS	*	NS
eicosatri-enoic	NS	NS	-[a]	NS	NS
arachidonic	NS	NS	*	*	NS
docosapent-aenoic	NS	NS	-	-	NS
docosahex-aenoic	NS	NS	***	*	-

The results refer to the data shown in Tables 66, 67.
[a]- notation as in Table 62b.
Significance of difference- notations as in Table 62a.

Table 68b The levels of significance for the changes in relative concentrations of the major fatty acids components in the triglycerides of the brain mitochondria between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	30.97 ±1.51	29.60 ±1.11	36.69 ±0.89	27.86 ±0.88	35.40 ±1.42	31.65 ±0.87
palmitoleic	2.79 ±1.18	2.40 ±1.04	2.88 ±1.19	2.46 ±0.72	4.95 ±0.16	5.27 ±0.36
stearic	8.67 ±0.56	8.96 ±0.61	8.44 ±0.26	9.71 ±0.73	13.58 ±0.46	13.36 ±0.80
oleic	35.13 ±2.12	34.31 ±0.50	25.94 ±0.62	31.68 ±1.20	37.38 ±1.13	42.93 ±0.80
linoleic	8.37 ±0.35	10.33 ±0.51	7.29 ±0.25	14.58 ±0.80	6.69 ±0.66	5.85 ±0.31
linolenic	0.37 ±0.13	0.28 ±0.08	Tr [a]	0.75 ±0.10	0.69 ±0.23	0.85 ±0.12
eicosatri-enoic	Tr	Tr	Tr	0.53 ±0.20	ND[b]	ND
arachidonic	3.09 ±0.40	3.56 ±0.43	2.48 ±0.36	3.97 ±0.60	Tr	Tr
docosapent-aenoic	0.29 ±0.05	0.30 ±0.13	0.68 ±0.18	1.82 ±0.30	0.66 ±0.60	ND
docosahex-aenoic	10.33 ±1.03	10.30 ±0.74	15.52 ±0.80	6.61 ±0.61	0.56 ±0.33	ND

Number of replicates as for Table 60.
[a,b]- notations as in Table 60.

Table 69 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the liver microsomes of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	30.44 ±0.62	NA[c]	33.05 ±1.00	31.14 ±1.08	33.61 ±0.77	28.80 ±3.56
palmitoleic	3.44 ±0.28	NA	2.25 ±0.15	1.35 ±0.37	7.86 ±0.35	6.28 ±0.57
stearic	11.81 ±0.37	NA	11.09 ±0.53	10.75 ±0.53	9.88 ±0.41	9.68 ±0.49
oleic	28.48 ±0.81	NA	27.16 ±0.48	33.26 ±0.96	41.01 ±0.40	44.85 ±1.80
linoleic	6.91 ±0.58	NA	8.00 ±0.50	13.67 ±0.79	6.61 ±0.68	8.40 ±2.15
linolenic	0.96 ±0.18	NA	0.28 ±0.17	0.50 ±0.09	0.91 ±0.09	0.94 ±0.28
eicosatri-enoic	Tr [a]	NA	ND[b]	ND	ND	0.22 ±0.14
arachidonic	4.10 ±0.28	NA	3.79 ±0.25	3.74 ±0.35	0.13 ±0.09	0.27 ±0.14
docosapent-aenoic	ND	ND	ND	0.43 ±0.26	ND	0.20 ±0.12
docosahex-aenoic	13.86 ±1.16	NA	14.36 ±1.08	5.16 ±0.80	ND	0.37 ±0.18

Number of replicates as for Table 61.
[a,b,c]- notations as in Tables 60, 61 respectively.

Table 70 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the liver microsomes of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	*	***	**	*
	(Y)[b]	NS	NS	NS	NS
palmitoleic	(M)	NS	NS	*	*
	(Y)	**	NS	***	***
stearic	(M)	NS	NS	**	*
	(Y)	NS	NS	NS	NS
oleic	(M)	**	**	*	***
	(Y)	NS	**	***	**
linoleic	(M)	*	***	***	***
	(Y)	NS	***	***	NS
linolenic	(M)	*	***	NS	NS
	(Y)	*	NS	*	NS
eicosatri-enoic	(M)	NS	-[c]	-	-
	(Y)	NS	NS	NS	-
arachidonic	(M)	NS	NS	***	***
	(Y)	NS	NS	***	***
docosapent-aenoic	(M)	NS	*	NS	*
	(Y)	NS	-	-	NS
docosahex-aenoic	(M)	**	***	***	***
	(Y)	NS	***	***	**

The results refer to the data shown in Tables 69, 70.
[a,b,c]- notations as in Table 62a.
Significance of difference- notations as in Table 62a.

Table 71a The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the liver microsomes during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	*	NS	NS	NS
palmitoleic	NS	NS	NS	***	NS
stearic	**	**	NS	***	**
oleic	*	NS	NS	*	NS
linoleic	NS	NS	NS	NS	NS
linolenic	*	NS	NS	NS	NS
eicosatri-enoic	NS	NS	-[a]	NS	-
arachidonic	NS	*	NS	-	-
docosapent-aenoic	-	**	*	-	-
docosahex-aenoic	NS	NS	NS	-	NS

The data refers to the results shown in Tables 69, 70
[a]- notation as in Table 62b.
Significance of difference- notations as in Table 62a.

Table 71b The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the liver microsomes between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	33.28 ±2.53	28.22 ±1.76	32.77 ±1.64	27.92 ±0.83	29.58 ±1.36	28.36 ±1.25
palmitoleic	6.07 ±0.59	14.09 ±4.54	11.88 ±1.39	4.76 ±0.77	8.48 ±0.68	7.22 ±0.35
stearic	12.43 ±1.16	10.62 ±2.14	8.60 ±0.44	12.28 ±2.39	6.54 ±1.26	8.39 ±0.57
oleic	31.38 ±1.36	28.52 ±0.99	31.19 ±0.97	37.41 ±0.98	35.94 ±2.08	39.58 ±1.05
linoleic	8.75 ±1.04	10.94 ±1.38	11.42 ±0.91	14.35 ±1.44	16.59 ±2.56	13.63 ±0.94
linolenic	0.83 ±0.20	0.77 ±0.13	0.90 ±0.49	0.53 ±0.21	1.81 ±0.17	2.44 ±0.93
eicosatri-enoic	1.02 ±0.58	0.30 ±0.17	Tr [a]	0.77 ±0.45	0.13 ±0.10	0.31 ±0.22
arachidonic	2.62 ±0.19	2.29 ±0.56	1.77 ±0.72	1.59 ±0.40	0.13 ±0.10	0.31 ±0.22
docosapent-aenoic	1.02 ±0.03	0.68 ±0.04	ND[b]	Tr	0.42 ±0.16	Tr
docosahex-aenoic	4.80 ±0.98	3.57 ±0.89	0.46 ±0.28	0.33 ±0.12	0.34 ±0.14	Tr

Number of replicates as for Table 63.
[a,b]- notations as in Table 60.

Table 72 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the heart microsomes of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	34.21 ±1.29	NA[c]	36.82 ±2.64	33.39 ±3.56	28.47 ±1.18	25.15 ±0.18
palmitoleic	4.21 ±0.32	NA	3.66 ±1.30	2.76 ±1.70	7.21 ±0.36	7.32 ±0.49
stearic	12.52 ±1.24	NA	11.35 ±2.00	10.77 ±1.73	6.40 ±0.37	7.61 ±0.11
oleic	34.24 ±1.36	NA	32.82 ±1.35	37.68 ±1.33	39.29 ±0.87	41.44 ±0.19
linoleic	9.78 ±1.24	NA	8.87 ±2.24	11.92 ±1.32	16.77 ±1.29	15.97 ±0.73
linolenic	0.32 ±0.14	NA	0.43 ±0.15	0.37 ±0.21	1.54 ±0.33	2.26 ±0.06
eicosatri-enoic	ND[b]	NA	ND	ND	ND	Tr [a]
arachidonic	2.67 ±1.00	NA	2.91 ±1.00	2.05 ±1.20	Tr	Tr
docosapent-aenoic	0.14 ±0.12	NA	0.21 ±0.13	Tr	Tr	Tr
docosahex-aenoic	1.94 ±0.21	NA	2.92 ±1.42	1.00 ±0.39	0.40 ±0.27	Tr

Number of replicates as for Table 64.
[a,b,c]- notations as in Tables 60, 61 respectively.

Table 73 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the heart microsomes of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	*	NS	NS
	(Y)[b]	NS	NS	NS	NS
palmitoleic	(M)	**	**	*	*
	(Y)	NS	NS	*	*
stearic	(M)	*	NS	NS	NS
	(Y)	NS	NS	*	NS
oleic	(M)	NS	**	NS	NS
	(Y)	NS	*	NS	*
linoleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	*	*
linolenic	(M)	NS	NS	**	NS
	(Y)	NS	NS	*	***
eicosatri-enoic	(M)	-[c]	-	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	NS	NS	-
	(Y)	NS	NS	-	-
docosapent-aenoic	(M)	***	NS	-	NS
	(Y)	NS	-	NS	NS
docosahex-aenoic	(M)	**	NS	NS	-
	(Y)	NS	NS	NS	*

The results refer to the data shown in Tables 73, 74.
[a,b,c]- notations as in Table 62a.
Significance of difference- notations as in Table 62a.

Table 74a The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the heart microsomes during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	*
palmitoleic	*	**	NS	NS	NS
stearic	NS	NS	NS	NS	NS
oleic	NS	NS	NS	NS	NS
linoleic	NS	NS	NS	NS	NS
linolenic	NS	NS	NS	NS	NS
eicosatri-enoic	NS	NS	-[a]	-	-
arachidonic	NS	NS	NS	-	NS
docosapent-aenoic	***	-	NS	-	NS
docosahex-aenoic	*	NS	NS	NS	NS

The results refer to the data shown in Tables 73, 74.
[a]- notation as in Table 62b.
Significance of difference- notations as in Table 62a.

Table 74b The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the heart microsomes between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	43.73 ±0.32	49.92 ±1.16	33.25 ±1.81	30.33 ±0.87	35.82 ±0.64	34.72 ±0.88
palmitoleic	6.14 ±0.86	9.18 ±0.93	4.71 ±0.41	4.59 ±1.12	5.16 ±0.43	5.15 ±0.90
stearic	10.58 ±0.79	10.93 ±0.68	13.32 ±2.03	10.10 ±1.10	14.21 ±0.96	13.75 ±1.01
oleic	25.74 ±0.68	25.90 ±0.44	34.37 ±1.23	37.41 ±1.32	31.54 ±1.32	34.46 ±1.37
linoleic	3.64 ±0.27	3.69 ±1.30	8.13 ±1.72	11.93 ±2.30	10.50 ±1.74	8.38 ±1.66
linolenic	0.49 ±0.29	0.40 ±0.23	0.92 ±0.21	1.00 ±0.38	1.27 ±0.28	1.11 ±0.26
eicosatri-enoic	1.01 ±0.36	0.31 ±0.22	0.41 ±0.25	0.14 ±0.07	Tr [a]	0.25 ±0.14
arachidonic	2.51 ±0.90	3.08 ±0.33	1.97 ±0.54	2.94 ±0.72	0.82 ±0.19	1.42 ±0.30
docosapent-aenoic	0.15 ±0.09	Tr	0.23 ±0.17	Tr	ND[b]	ND
docosahex-aenoic	5.82 ±0.28	4.66 ±0.37	1.91 ±0.76	1.62 ±0.15	0.61 ±0.23	0.75 ±0.29

Number of replicates as for Table 66.
[a,b]- notations as in Table 60.

Table 75 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the brain microsomes of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	44.86 ±1.23	NA[c]	42.81 ±1.67	43.85 ±2.51	34.54 ±0.89	31.39 ±1.48
palmitoleic	6.79 ±0.62	NA	3.57 ±0.48	6.11 ±1.08	4.91 ±1.47	6.93 ±1.17
stearic	13.24 ±2.14	NA	17.37 ±1.64	15.43 ±2.25	12.81 ±1.09	19.85 ±1.44
oleic	26.42 ±1.23	NA	27.68 ±1.95	27.30 ±0.93	36.94 ±2.08	38.18 ±2.33
linoleic	3.79 ±0.34	NA	5.11 ±1.32	3.79 ±0.42	9.98 ±0.78	11.95 ±1.90
linolenic	Tr [a]	NA	ND[b]	Tr	ND	ND
eicosatri-enoic	ND	NA	ND	Tr	ND	ND
arachidonic	3.13 ±0.22	NA	2.66 ±0.32	2.55 ±1.24	0.24 ±0.15	ND
docosapent-aenoic	ND	NA	ND	ND	ND	ND
docosahex-aenoic	2.15 ±0.22	NA	0.88 ±0.09	0.94 ±0.10	0.16 ±0.21	ND

Number of replicates as for Table 67.
[a,b,c]- notations as in Tables 60, 61 respectively.

Table 76 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the brain microsomes of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	**	NS	**	*
	(Y)[b]	NS	NS	*	**
palmitoleic	(M)	NS	NS	NS	NS
	(Y)	**	NS	NS	NS
stearic	(M)	NS	NS	*	NS
	(Y)	NS	NS	NS	NS
oleic	(M)	***	NS	*	NS
	(Y)	NS	NS	**	**
linoleic	(M)	*	NS	NS	NS
	(Y)	NS	NS	***	**
linolenic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
eicosatri-enoic	(M)	NS	NS	-[c]	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	NS	*	NS
	(Y)	NS	NS	NS	-
docosapent-aenoic	(M)	NS	-	NS	NS
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	**	NS	*	*
	(Y)	**	NS	*	***

The results refer to the data shown in Tables 75, 76.
[a,b,c]- notations as in Table 62a.
Significance of difference- notations as in Table 62a.

Table 77a The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the brain microsomes during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	**	**	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	NS	NS	*
oleic	NS	*	***	NS	NS
linoleic	NS	NS	*	NS	NS
linolenic	-[a]	**	*	NS	NS
eicosatri-enoic	*	-	-	NS	-
arachidonic	NS	NS	NS	NS	**
docosapent-aenoic	-	-	NS	NS	NS
docosahex-aenoic	***	NS	**	NS	*

The results refer to the data shown in Tables 75, 76.
[a]- notation as in Table 62b.
Significance of difference- notations as in Table 62a.

Table 77b The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the brain microsomes between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	26.25 ±1.52	31.50 ±4.50	26.90 ±1.24	23.89 ±0.89	33.57 ±0.12	29.81 ±3.25
palmitoleic	2.43 ±1.30	2.93 ±0.41	1.40 ±0.91	2.52 ±0.23	6.89 ±0.58	6.63 ±0.95
stearic	7.64 ±0.23	7.51 ±0.76	7.76 ±0.45	9.66 ±0.38	13.12 ±1.84	15.28 ±2.11
oleic	38.47 ±2.11	36.90 ±1.09	41.34 ±1.09	42.39 ±0.68	38.42 ±1.94	43.02 ±0.61
linoleic	10.51 ±0.62	9.32 ±2.18	12.18 ±0.69	14.18 ±0.14	6.76 ±0.04	4.25 ±0.42
linolenic	0.30 ±0.05	0.12 ±0.06	0.51 ±0.03	0.94 ±0.11	0.72 ±0.32	0.81 ±0.19
eicosatri-enoic	ND[b]	ND[c]	Tr [a]	Tr	ND	ND
arachidonic	3.09 ±0.27	6.17 ±1.68	2.19 ±0.20	2.25 ±0.34	Tr	Tr
docosapent-aenoic	0.37 ±0.02	0.18 ±0.15	0.40 ±0.20	0.50 ±0.20	Tr	ND
docosahex-aenoic	10.94 ±0.98	5.37 ±2.52	7.32 ±0.51	3.60 ±0.29	0.41 ±0.06	ND

Number of replicates as for Table 60.
[a,b]- notations as in Table 60.

Table 78 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the liver supernatant of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	22.71 ±1.22	NA[b]	27.27 ±1.21	27.43 ±0.18	32.26 ±0.35	29.16 ±4.96
palmitoleic	2.70 ±0.24	NA	2.20 ±0.11	2.04 ±0.39	9.06 ±1.08	6.44 ±1.70
stearic	9.71 ±0.41	NA	10.63 ±0.43	11.05 ±0.84	8.10 ±0.97	9.43 ±0.21
oleic	32.29 ±0.52	NA	39.34 ±0.34	40.98 ±0.26	43.85 ±0.62	46.74 ±2.46
linoleic	9.54 ±0.64	NA	9.81 ±0.46	12.18 ±0.05	5.56 ±0.09	6.55 ±1.11
linolenic	0.78 ±0.24	NA	0.64 ±0.15	0.47 ±0.14	0.96 ±0.08	1.20 ±0.47
eicosatri-enoic	0.35 ±0.21	NA	1.07 ±0.66	0.32 ±0.12	Tr [a]	Tr
arachidonic	7.34 ±0.35	NA	2.20 ±0.35	1.97 ±0.03	Tr	0.12 ±0.08
docosapent-aenoic	0.59 ±0.17	NA	0.23 ±0.13	0.40 ±0.24	Tr	0.14 ±0.12
docosahex-aenoic	14.00 ±0.87	NA	7.06 ±0.68	3.18 ±0.58	0.15 ±0.03	0.22 ±0.12

Number of replicates as for Table 61.
[a]- notation as in Table 60.
[b]= no samples available due to high embryo mortality.

Table 79 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the liver supernatant of embryos and post-hatch chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	***	NS
	(Y)[b]	*	NS	**	NS
palmitoleic	(M)	NS	NS	***	**
	(Y)	NS	NS	***	*
stearic	(M)	NS	*	NS	*
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	NS	NS	NS
	(Y)	***	NS	**	NS
linoleic	(M)	NS	*	***	***
	(Y)	NS	**	***	**
linolenic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	*	NS
eicosatri-enoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	*	NS	***	***
	(Y)	***	NS	***	***
docosapent-aenoic	(M)	NS	NS	-[c]	-
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	*	NS	NS	*
	(Y)	***	**	**	**

The results refer to the data shown in Tables 78, 79.
[a,b,c]- notations as in Table 62a.
Significance of difference- notations as in Table 62a.

Table 80a The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the liver supernatant during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	**	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	**	**	NS	NS	*
oleic	*	NS	NS	*	NS
linoleic	NS	*	***	***	NS
linolenic	NS	NS	NS	NS	NS
eicosatri-enoic	-[a]	-	-	NS	NS
arachidonic	***	NS	NS	NS	NS
docosapent-aenoic	NS	NS	NS	NS	-
docosahex-aenoic	NS	NS	NS	**	-

The results refer to the data shown in Tables 78, 79.
[a]- notation as in Table 62b
Significance of difference- notations as in Table 62a.

Table 80b The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the liver supernatant between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	33.05 ±0.77	30.22 ±2.72	29.34 ±0.62	26.05 ±0.96	25.70 ±2.75	25.62 ±0.45
palmitoleic	4.20 ±1.57	3.92 ±0.07	5.22 ±1.64	3.43 ±0.26	8.66 ±1.78	6.24 ±0.27
stearic	14.26 ±1.55	12.79 ±1.77	10.05 ±0.24	8.28 ±0.43	9.15 ±0.78	8.30 ±0.31
oleic	29.28 ±2.48	35.94 ±2.68	35.04 ±3.92	42.90 ±0.66	37.58 ±2.04	43.14 ±0.51
linoleic	4.98 ±2.14	8.89 ±3.27	14.27 ±0.44	15.40 ±0.35	16.34 ±2.61	14.56 ±1.25
linolenic	0.41 ±0.23	0.63 ±0.39	0.48 ±0.25	0.58 ±0.10	1.57 ±0.03	1.22 ±1.01
eicosatri-enoic	ND[b]	0.19 ±0.05	ND	0.35 ±0.24	0.34 ±0.31	0.18 ±0.01
arachidonic	5.31 ±1.52	2.95 ±1.67	2.47 ±0.59	1.56 ±0.33	0.16 ±0.04	Tr [a]
docosapent-aenoic	0.84 ±0.44	0.32 ±0.12	0.40 ±0.10	0.36 ±0.18	0.21 ±0.02	0.28 ±0.02
docosahex-aenoic	7.84 ±1.05	4.35 ±2.04	2.83 ±0.83	1.10 ±1.06	0.24 ±0.01	0.35 ±0.10

Number of replicates as for Table 63.
[a,b]- notations as in Table 60.

Table 81 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the heart supernatant of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	33.12 ±0.67	NA[c]	32.14 ±1.91	30.12 ±3.04	26.26 ±1.06	24.89 ±0.31
palmitoleic	4.12 ±0.62	NA	5.83 ±2.49	3.94 ±0.58	6.73 ±0.15	6.27 ±0.88
stearic	13.59 ±1.62	NA	13.85 ±1.16	9.88 ±1.14	7.27 ±0.37	7.62 ±0.26
oleic	26.02 ±1.62	NA	28.77 ±1.54	44.41 ±4.40	39.51 ±1.15	42.23 ±0.42
linoleic	8.17 ±1.38	NA	14.57 ±4.82	9.47 ±0.46	17.57 ±0.96	15.69 ±0.79
linolenic	0.42 ±0.32	NA	Tr [a]	2.02 ±0.02	2.18 ±0.16	2.20 ±0.06
eicosatri-enoic	ND[b]	NA	ND	ND	Tr	0.52 ±0.34
arachidonic	6.24 ±1.23	NA	4.55 ±0.98	1.17 ±0.43	Tr	0.34 ±0.14
docosapent-aenoic	0.52 ±0.31	NA	0.36 ±0.16	Tr	Tr	Tr
docosahex-aenoic	7.84 ±1.24	NA	4.04 ±0.83	0.58 ±0.26	0.35 ±0.14	0.22 ±0.03

Number of replicates as for Table 64.
[a,b,c]- notations as in Tables 60, 61 respectively.

Table 82 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the heart supernatant of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	**	*	NS	NS
	(Y)[b]	NS	NS	NS	NS
palmitoleic	(M)	NS	NS	*	***
	(Y)	NS	NS	**	NS
stearic	(M)	*	*	NS	NS
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	NS	NS	NS
	(Y)	NS	*	NS	NS
linoleic	(M)	**	NS	NS	NS
	(Y)	NS	NS	***	***
linolenic	(M)	NS	NS	***	NS
	(Y)	-	-	***	***
eicosatri-enoic	(M)	NS	-[c]	NS	NS
	(Y)	NS	NS	NS	-
arachidonic	(M)	NS	NS	**	**
	(Y)	NS	*	*	NS
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	-	NS	NS
docosahex-aenoic	(M)	**	NS	NS	NS
	(Y)	*	**	NS	NS

The data refers to the results shown in Tables 81, 82.
[a,b,c]- notations as in Table 62a.
Significance of difference- notations as in Table 62a.

Table 83a The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the heart supernatant during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	*	NS	NS	NS
oleic	NS	NS	NS	NS	NS
linoleic	NS	NS	***	NS	NS
linolenic	NS	-[a]	*	**	NS
eicosatri-enoic	NS	NS	-	-	NS
arachidonic	NS	NS	NS	-	-
docosapent-aenoic	NS	NS	-	-	-
docosahex-aenoic	NS	NS	NS	NS	*

The data refers to the results shown in Tables 81, 82.
[a]- notation as in Table 62b.
Significance of difference- notations as in Table 62a.

Table 83b The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the heart supernatant between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	24.01 ±2.83	32.91 ±1.82	32.94 ±4.94	35.42 ±4.53	35.20 ±1.91	38.56 ±1.21
palmitoleic	4.94 ±1.26	6.70 ±1.50	7.60 ±0.57	4.00 ±1.26	6.55 ±1.36	8.10 ±0.65
stearic	9.20 ±0.60	10.29 ±0.82	9.05 ±0.51	9.38 ±1.74	11.62 ±3.28	13.20 ±0.39
oleic	32.18 ±2.36	31.75 ±1.74	32.71 ±0.49	36.26 ±1.86	34.07 ±1.35	32.89 ±1.24
linoleic	7.73 ±1.11	7.11 ±0.19	12.78 ±2.79	12.94 ±3.67	10.20 ±2.90	7.26 ±0.30
linolenic	0.81 ±0.11	1.06 ±0.06	1.62 ±0.60	0.59 ±0.29	0.63 ±0.23	ND
eicosatri-enoic	Tr [a]	1.64 ±0.94	ND[b]	0.11 ±0.01	ND	ND
arachidonic	2.25 ±0.57	5.97 ±2.18	1.20 ±0.07	0.72 ±0.42	0.35 ±0.15	ND
docosapent-aenoic	0.67 ±0.16	1.11 ±0.80	Tr	ND	Tr	ND
docosahex-aenoic	8.12 ±0.90	1.46 ±0.96	1.71 ±0.01	0.58 ±0.28	1.27 ±0.97	ND

Number of replicates as for Table 66.
[a,b]- notations as in Table 60.

Table 84 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the brain supernatant of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	41.24 ±0.52	NA[c]	41.31 ±0.40	42.52 ±2.13	33.34 ±1.38	33.05 ±1.05
palmitoleic	5.12 ±0.43	NA	7.23 ±0.10	3.18 ±0.20	8.35 ±0.95	8.55 ±1.45
stearic	8.12 ±0.62	NA	8.93 ±1.29	7.78 ±0.52	8.50 ±2.33	9.28 ±0.91
oleic	32.84 ±1.24	NA	31.94 ±1.64	33.05 ±2.10	36.62 ±0.43	40.35 ±3.66
linoleic	7.21 ±1.06	NA	5.90 ±0.32	7.00 ±1.06	11.44 ±2.62	6.24 ±2.04
linolenic	0.38 ±0.21	NA	0.28 ±0.04	0.64 ±0.18	1.37 ±0.10	1.68 ±0.12
eicosatri-enoic	Tr [a]	NA	Tr	Tr	Tr	Tr
arachidonic	3.21 ±0.47	NA	1.36 ±0.55	2.00 ±0.78	0.45 ±0.15	0.45 ±0.23
docosapent-aenoic	Tr	NA	ND[b]	1.60 ±0.40	0.36 ±0.16	Tr
docosahex-aenoic	1.88 ±0.32	NA	3.12 ±0.14	2.13 ±0.22	Tr	0.20 ±0.01

Number of replicates, as for Table 67.
[a,b,c]- notations as in Tables 60, 61 respectively.

Table 85 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the triglycerides of the brain supernatant of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	NS	NS
	(Y)[b]	NS	NS	*	**
palmitoleic	(M)	NS	NS	NS	*
	(Y)	**	***	**	*
stearic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
linoleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
linolenic	(M)	NS	NS	NS	-
	(Y)	NS	NS	*	**
eicosatri-enoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	NS	NS	-[c]
	(Y)	*	NS	NS	NS
docosapent-aenoic	(M)	*	NS	NS	NS
	(Y)	NS	**	*	**
docosahex-aenoic	(M)	***	**	NS	-
	(Y)	*	**	***	***

The results refer to the data shown in Tables 84, 85.
[a,b,c]- notations as in Table 62a.
Significance of difference- notations as in Table 62a.

Table 86a The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the brain supernatant during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	***	NS	NS	NS	*
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	NS	NS	**
oleic	NS	NS	NS	NS	NS
linoleic	NS	*	NS	NS	NS
linolenic	NS	NS	NS	*	***
eicosatri-enoic	NS	NS	-[a]	NS	NS
arachidonic	NS	NS	NS	NS	NS
docosapent-aenoic	**	NS	**	-	NS
docosahex-aenoic	***	***	**	-	-

The results refer to the data shown in Tables 84, 85.
[a]- notation as in Table 62b.
Significance of difference- notations as in Table 62a.

Table 86b The levels of significance for the changes in relative concentrations of the major fatty acid components in the triglycerides of the brain supernatant between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	19.07 ±4.68	9.78 ±1.40	14.77 ±2.68	6.10 ±0.42	12.69 ±2.80	18.41 ±4.63
palmitoleic	8.70 ±2.29	4.54 ±0.71	5.48 ±1.68	1.69 ±0.61	2.80 ±1.21	2.02 ±1.18
stearic	5.13 ±1.25	3.01 ±0.30	5.33 ±0.58	3.27 ±0.38	5.96 ±1.31	11.89 ±2.43
oleic	58.34 ±4.87	70.05 ±1.92	58.88 ±2.42	71.65 ±1.07	69.72 ±3.34	53.00 ±4.81
linoleic	6.42 ±0.92	10.26 ±1.04	9.29 ±0.97	13.72 ±0.44	7.20 ±0.38	13.86 ±1.08
linolenic	0.73 ±0.41	Tr [a]	ND[b]	0.18 ±0.09	0.36 ±0.21	ND
eicosatri-enoic	ND	ND	ND	ND	ND	ND
arachidonic	0.76 ±0.35	1.14 ±0.18	4.73 ±0.57	2.24 ±0.29	ND	ND
docosapent-aenoic	ND	ND	ND	ND	ND	ND
docosahex-aenoic	1.68 ±1.19	1.22 ±0.29	1.42 ±0.28	1.15 ±0.37	1.27 ±0.74	1.32 ±0.83

The number of replicates used for analysis was- embryos 5, chicks 4.
[a]= relative concentration less than 0.1 per cent of the total fatty acids identified.
[b]= no detected concentration.

Table 87 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the liver mitochondria of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	10.58 ±0.21	NA[c]	5.57 ±0.38	7.08 ±0.28	12.65 ±1.73	15.96 ±2.94
palmitoleic	5.34 ±0.61	NA	1.90 ±0.36	1.62 ±0.24	5.57 ±0.56	4.24 ±0.16
stearic	3.82 ±0.65	NA	4.82 ±0.23	3.98 ±0.14	5.78 ±1.18	7.22 ±1.25
oleic	62.64 ±0.21	NA	65.66 ±1.73	69.44 ±0.38	65.67 ±2.95	61.17 ±2.76
linoleic	8.98 ±0.12	NA	13.14 ±2.59	14.57 ±0.27	9.32 ±1.43	10.57 ±2.19
linolenic	0.78 ±0.14	NA	0.30 ±0.18	0.32 ±0.18	0.55 ±0.32	0.45 ±0.26
eicosatri-enoic	Tr [a]	NA	ND[b]	ND	ND	ND
arachidonic	3.64 ±0.39	NA	4.80 ±1.18	2.17 ±0.14	ND	0.40 ±0.23
docosapent-aenoic	Tr	NA	ND	ND	ND	ND
docosahex-aenoic	3.65 ±0.44	NA	3.62 ±0.59	0.82 ±0.48	0.46 ±0.31	ND

The number of replicates used for all analyses was 4.
[a,b]-notations as in Table 87.
[c]= no samples available due to high embryo mortality.

Table 88 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the liver mitochondria of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	*	NS	*
	(Y)[b]	***	*	*	*
palmitoleic	(M)	NS	NS	NS	NS
	(Y)	**	NS	***	***
stearic	(M)	NS	*	NS	*
	(Y)	NS	*	NS	*
oleic	(M)	NS	**	NS	**
	(Y)	NS	NS	NS	*
linoleic	(M)	NS	**	***	NS
	(Y)	NS	NS	*	NS
linolenic	(M)	-[c]	-	NS	-
	(Y)	NS	NS	NS	NS
eicosatri-enoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	***	**	***	***
	(Y)	NS	NS	***	***
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	**	NS	-

The results refer to the data shown in Tables 87, 88.
[a]= results for progeny from mature parents (thirty-seven to forty weeks of age).
[b]= results for progeny from young parents (twenty-three to twenty-four weeks of age).
[c]= values too low to allow accurate comparisons.

Significance of difference: *=P<0.05 **=P<0.01 ***=P<0.001
NS= Not significant

Table 89a The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the liver mitochondria during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	*	NS	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	NS	NS	NS
oleic	NS	NS	NS	NS	NS
linoleic	*	NS	NS	NS	NS
linolenic	NS	-[a]	NS	NS	-
eicosatri-enoic	NS	NS	NS	NS	NS
arachidonic	***	NS	NS	NS	-
docosapent-aenoic	NS	NS	NS	NS	NS
docosahex-aenoic	NS	*	NS	NS	-

The results refer to the data shown in Tables 87, 88
[a]= values too low to allow accurate comparisons.
Significance of difference- notations as in Table 89a.

Table 89b The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the liver mitochondria between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	18.73 ±3.43	19.78 ±1.55	17.84 ±2.77	15.33 ±1.91	25.20 ±1.06	34.93 ±4.13
palmitoleic	6.42 ±1.75	11.09 ±2.34	9.70 ±2.82	4.57 ±1.86	12.56 ±0.89	3.73 ±0.63
stearic	7.53 ±2.16	6.64 ±0.48	4.88 ±0.62	5.15 ±0.91	8.72 ±0.88	21.07 ±3.14
oleic	53.54 ±2.00	44.14 ±3.30	49.41 ±1.60	51.17 ±2.00	42.56 ±0.66	28.85 ±3.21
linoleic	9.13 ±2.66	14.10 ±0.43	10.81 ±2.29	18.50 ±1.49	10.41 ±0.38	11.47 ±4.69
linolenic	ND[b]	1.22 ±0.76	0.65 ±0.26	0.88 ±0.63	Tr [a]	ND
eicosatri-enoic	Tr	Tr	1.36 ±0.74	Tr	Tr	ND
arachidonic	1.86 ±0.55	2.10 ±0.35	2.77 ±0.16	3.50 ±0.16	ND	ND
docosapent-aenoic	Tr	Tr	0.18 ±0.10	Tr	ND	ND
docosahex-aenoic	2.66 ±0.95	0.93 ±0.54	2.40 ±0.96	0.90 ±0.52	0.49 ±0.24	ND

The number of replicates used for all analyses was 4.
[a,b]- notations as in Table 87.

Table 90 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the heart mitochondria of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	17.12 ±1.23	NA[c]	16.34 ±1.13	12.38 ±0.48	14.80 ±2.64	34.08 ±3.49
palmitoleic	6.21 ±1.32	NA	4.99 ±2.00	2.38 ±0.56	6.96 ±1.17	7.48 ±1.74
stearic	6.23 ±1.24	NA	4.40 ±0.78	4.19 ±0.53	9.51 ±2.45	9.84 ±2.63
oleic	52.49 ±2.19	NA	51.24 ±0.38	57.86 ±2.30	48.67 ±2.55	33.65 ±3.38
linoleic	14.24 ±1.23	NA	16.06 ±0.70	18.44 ±0.78	18.48 ±2.67	14.96 ±2.16
linolenic	Tr [a]	NA	0.46 ±0.28	0.51 ±0.18	ND[b]	ND
eicosatri-enoic	ND	NA	ND	Tr	ND	ND
arachidonic	2.14 ±0.72	NA	4.86 ±0.62	3.00 ±1.10	1.71 ±1.09	ND
docosapent-aenoic	ND	NA	ND	Tr	ND	ND
docosahex-aenoic	1.57 ±0.67	NA	1.65 ±0.95	1.11 ±0.56	Tr	ND

The number of replicates used for all analyses was 4.
[a,b,c]- notations as in Tables 87, 88 respectively.

Table 91 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the heart mitochondria of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	**	**
	(Y)[b]	NS	*	NS	***
palmitoleic	(M)	NS	NS	**	NS
	(Y)	NS	NS	*	*
stearic	(M)	NS	NS	*	**
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	NS	**	**
	(Y)	NS	*	*	**
linoleic	(M)	NS	*	**	NS
	(Y)	NS	NS	NS	NS
linolenic	(M)	*	NS	-[c]	-
	(Y)	-	NS	*	*
eicosatri-enoic	(M)	-	-	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	*	***	***
	(Y)	*	NS	NS	*
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	NS	NS	NS	-
	(Y)	NS	NS	-	-

The results refer to the data shown in Tables 90, 91.
[a,b,c]- notations as in Table 89a.
Significance of difference- notations as in Table 89a.

Table 92a The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the heart mitochondria during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	*	NS
palmitoleic	NS	NS	NS	**	NS
stearic	NS	NS	NS	NS	*
oleic	NS	NS	NS	NS	NS
linoleic	NS	NS	NS	*	NS
linolenic	NS	NS	NS	NS	NS
eicosatri-enoic	NS	-[a]	NS	NS	NS
arachidonic	NS	*	NS	-	NS
docosapent-aenoic	NS	NS	NS	NS	NS
docosahex-aenoic	NS	NS	NS	-	NS

The results refer to the data shown in Tables 90, 91.
[a]- notation as in Table 89b.
Significance of difference- notations as in Table 89a.

Table 92b The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the heart mitochondria between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	26.18 ±4.25	27.86 ±2.90	21.42 ±4.07	20.67 ±4.35	29.64 ±2.99	40.21 ±0.90
palmitoleic	7.14 ±1.99	11.70 ±3.50	3.92 ±1.68	1.98 ±1.29	9.18 ±2.04	5.27 ±2.32
stearic	12.33 ±2.70	8.56 ±1.49	6.30 ±1.12	14.55 ±2.54	13.45 ±2.54	19.38 ±1.36
oleic	40.14 ±3.37	41.83 ±7.17	41.77 ±4.32	49.59 ±9.15	40.78 ±0.44	32.20 ±1.35
linoleic	11.28 ±0.56	8.91 ±0.83	16.58 ±4.59	8.05 ±3.52	5.68 ±0.20	1.35 ±0.35
linolenic	ND[b]	1.15 ±0.12	2.88 ±0.61	ND	ND	ND
eicosatri-enoic	Tr [a]	ND	0.21 ±0.12	1.01 ±0.61	ND	ND
arachidonic	1.52 ±0.91	Tr	3.62 ±1.62	2.08 ±0.70	1.30 ±0.79	1.59 ±0.58
docosapent-aenoic	ND	ND	ND	ND	ND	ND
docosahex-aenoic	1.42 ±0.82	Tr	3.81 ±1.42	2.07 ±0.96	Tr	ND

The number of replicates used for all analyses was 4.
[a,b]- notations as in Table 87.

Table 93 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the brain mitochondria of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	25.24 ±2.36	NA[c]	27.60 ±1.36	24.39 ±1.37	29.61 ±1.52	37.57 ±0.87
palmitoleic	5.22 ±1.37	NA	2.22 ±0.84	3.67 ±0.63	5.32 ±0.16	5.48 ±1.94
stearic	13.24 ±2.10	NA	12.97 ±2.53	11.51 ±1.48	14.26 ±2.03	22.33 ±4.52
oleic	47.27 ±2.62	NA	47.84 ±2.54	49.25 ±1.50	45.30 ±0.87	29.19 ±2.54
linoleic	9.07 ±0.34	NA	9.37 ±0.97	7.62 ±0.33	5.22 ±0.30	4.04 ±1.39
linolenic	ND[b]	NA	ND	0.48 ±0.29	ND	ND
eicosatri-enoic	ND	NA	ND	ND	ND	ND
arachidonic	Tr [a]	NA	Tr	2.45 ±0.84	ND	1.40 ±0.92
docosapent-aenoic	ND	NA	ND	ND	ND	ND
docosahex-aenoic	Tr	NA	ND	0.63 ±0.37	ND	ND

The number of replicates used for all analyses was 4.
[a,b,c]- notations as in Tables 87, 88 respectively.

Table 94 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the brain mitochondria of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	NS	**
	(Y)[b]	NS	NS	*	***
palmitoleic	(M)	NS	NS	*	NS
	(Y)	NS	NS	*	NS
stearic	(M)	NS	*	NS	NS
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	***
linoleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	**	*
linolenic	(M)	**	**	NS	NS
	(Y)	NS	-[c]	-	-
eicosatri-enoic	(M)	NS	NS	-	-
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	NS	NS	NS
	(Y)	NS	*	*	NS
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	NS	NS	-	-
	(Y)	NS	-	-	-

The results refer to the data shown in Tables 93, 94.
[a,b,c]- notations as in Table 89a.
Significance of difference- notations as in Table 89a.

Table 95a The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the brain mitochondria during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	NS	NS	NS
oleic	NS	NS	NS	**	NS
linoleic	*	NS	NS	NS	NS
linolenic	NS	**	-[a]	NS	NS
eicosatri-enoic	NS	-	-	NS	NS
arachidonic	-	-	NS	-	NS
docosapent-aenoic	NS	NS	NS	NS	NS
docosahex-aenoic	-	*	NS	NS	NS

The results refer to the data shown in Tables 93, 94.
[a]- notation as in Table 89b.
Significance of difference- notations as in Table 89a.

Table 95b The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the brain mitochondria between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	9.69 ±1.18	6.42 ±0.92	7.68 ±0.84	7.00 ±1.59	18.37 ±4.91	28.49 ±4.81
palmitoleic	6.70 ±0.97	2.41 ±0.55	3.53 ±0.77	3.00 ±1.07	1.15 ±0.67	4.87 ±0.21
stearic	3.29 ±0.37	2.68 ±0.18	3.69 ±0.20	4.18 ±0.46	10.31 ±1.05	14.45 ±0.73
oleic	65.30 ±1.96	71.96 ±0.65	64.93 ±1.75	67.12 ±0.80	63.40 ±5.00	43.41 ±5.46
linoleic	9.21 ±1.57	13.77 ±1.28	13.31 ±1.43	13.87 ±2.07	5.04 ±0.81	8.79 ±1.36
linolenic	ND*	0.32 ±0.13	0.26 ±0.12	0.51 ±0.19	ND	ND
eicosatri-enoic	ND	ND	ND	ND	ND	ND
arachidonic	2.48 ±0.87	1.34 ±0.06	4.90 ±0.19	2.60 ±0.10	1.70 ±1.30	ND
docosapent-aenoic	ND	ND	ND	ND	ND	ND
docosahex-aenoic	3.33 ±0.65	1.18 ±0.27	1.66 ±0.10	1.71 ±0.42	ND	ND

Number of replicates as for Table 87.
*= no detected concentration.

Table 96 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the liver microsomes of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	11.36 ±1.18	NA[c]	5.04 ±0.64	6.41 ±1.08	18.14 ±1.13	21.49 ±3.23
palmitoleic	6.02 ±0.60	NA	1.45 ±0.17	1.30 ±0.22	3.90 ±1.14	3.90 ±0.34
stearic	4.03 ±0.79	NA	4.34 ±0.38	4.17 ±0.49	9.22 ±0.55	12.02 ±1.38
oleic	61.13 ±1.59	NA	70.49 ±0.41	70.11 ±1.99	57.76 ±2.18	53.06 ±3.75
linoleic	9.65 ±0.80	NA	13.55 ±0.19	14.37 ±0.73	9.71 ±0.87	9.54 ±1.22
linolenic	0.96 ±0.08	NA	0.39 ±0.23	0.45 ±0.15	ND	ND
eicosatri-enoic	ND	NA	ND[b]	ND	ND	ND
arachidonic	3.63 ±0.40	NA	3.26 ±0.34	2.33 ±0.40	0.77 ±0.44	ND
docosapent-aenoic	ND	NA	Tr [a]	ND	ND	ND
docosahex-aenoic	3.24 ±0.59	NA	1.40 ±0.82	0.87 ±0.50	0.50 ±0.29	ND

Number of replicates as for Table 88.
[a,b,c]- notations as in Tables 87, 88 respectively.

Table 97 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the liver microsomes of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	NS	**
	(Y)[b]	**	NS	***	**
palmitoleic	(M)	*	NS	NS	NS
	(Y)	***	NS	NS	***
stearic	(M)	NS	NS	**	***
	(Y)	NS	NS	***	**
oleic	(M)	NS	NS	NS	**
	(Y)	**	NS	**	**
linoleic	(M)	NS	NS	**	NS
	(Y)	**	NS	**	*
linolenic	(M)	-[c]	NS	*	*
	(Y)	NS	NS	*	*
eicosatri-enoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	*	***	NS	***
	(Y)	NS	NS	*	**
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	*	NS	**	**
	(Y)	NS	NS	NS	-

The results refer to the data shown in Tables 96, 97.
[a,b,c]- notations as in Table 89a.
Significance of difference- notations as in Table 89a.

Table 98a The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the liver microsomes during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	*	NS	NS	NS
palmitoleic	NS	*	NS	NS	NS
stearic	NS	NS	NS	NS	NS
oleic	NS	*	NS	NS	NS
linoleic	NS	NS	NS	**	NS
linolenic	***	NS	NS	NS	NS
eicosatri-enoic	NS	NS	NS	NS	NS
arachidonic	NS	**	NS	NS	NS
docosapent-aenoic	NS	NS	NS	NS	NS
docosahex-aenoic	NS	NS	NS	-[a]	NS

The results refer to the data shown in Tables 96, 97.
[a]- notation as in Table 89b.
Significance of difference- notations as in Table 89a.

Table 98b The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the liver microsomes between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	26.64 ±5.01	16.96 ±1.78	21.90 ±1.49	17.58 ±3.20	24.82 ±3.14	37.70 ±2.23
palmitoleic	9.43 ±1.72	7.65 ±2.03	10.68 ±2.44	7.32 ±3.43	11.37 ±3.96	11.28 ±1.92
stearic	7.27 ±0.74	7.40 ±1.12	5.75 ±1.32	7.81 ±1.62	9.04 ±2.67	15.03 ±1.89
oleic	43.40 ±2.47	51.13 ±2.53	46.16 ±3.73	50.18 ±4.44	46.79 ±5.75	28.00 ±2.30
linoleic	9.40 ±1.74	13.40 ±2.16	11.20 ±0.54	17.08 ±1.91	6.38 ±1.50	8.00 ±1.06
linolenic	Tr [a]	ND[b]	0.37 ±0.22	ND	ND	ND
eicosatri-enoic	0.83 ±0.48	ND	ND	ND	ND	ND
arachidonic	1.51 ±0.74	1.82 ±0.64	2.33 ±0.78	ND	0.55 ±0.35	ND
docosapent-aenoic	ND	ND	ND	ND	ND	ND
docosahex-aenoic	1.74 ±0.41	1.65 ±0.63	1.62 ±0.55	ND	1.06 ±0.82	ND

Number of replicates as for Table 90.
[a,b]- notations as in Table 87.

Table 99 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the heart microsomes of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	9.24 ±1.23	NA[c]	5.65 ±3.55	18.62 ±2.19	19.67 ±4.39	30.52 ±0.28
palmitoleic	6.24 ±1.23	NA	5.50 ±3.20	2.18 ±1.27	8.28 ±1.11	5.87 ±1.66
stearic	10.42 ±1.27	NA	10.37 ±2.74	8.56 ±2.34	8.94 ±1.84	12.42 ±3.35
oleic	60.14 ±3.21	NA	63.01 ±5.60	52.30 ±5.09	47.08 ±2.57	31.26 ±0.49
linoleic	12.92 ±0.62	NA	13.29 ±0.75	16.24 ±0.48	16.10 ±2.68	19.32 ±0.75
linolenic	ND[b]	NA	ND	ND	ND	0.59 ±0.32
eicosatri-enoic	ND	NA	ND	ND	ND	ND
arachidonic	1.04 ±0.24	NA	2.10 ±1.49	0.98 ±0.69	Tr [a]	ND
docosapent-aenoic	ND	NA	ND	ND	ND	ND
docosahex-aenoic	Tr	NA	Tr	1.30 ±0.98	ND	ND

Number of replicates as for Table 91.
[a,b,c]- notations as in Tables 87, 88 respectively.

Table 100 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the heart microsomes of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	NS	**
	(Y)[b]	NS	*	NS	**
palmitoleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	*	NS
stearic	(M)	NS	NS	NS	*
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	NS	NS	**
	(Y)	NS	NS	NS	**
linoleic	(M)	NS	*	**	**
	(Y)	NS	*	NS	*
linolenic	(M)	-[c]	-	NS	NS
	(Y)	NS	NS	NS	-
eicosatri-enoic	(M)	-	NS	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	*	-	NS
	(Y)	NS	NS	-	-
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	NS	*	-	NS
	(Y)	NS	-	-	-

The results refer to the data shown in Tables 99, 100.
[a,b,c]- notations as in Table 89a.
Significance of difference- notations as in Table 89a.

Table 101a The levels of significance for the changes in relative concentrations of the major fatty acids in the cholesteryl esters of the heart microsomes during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	*	**	NS	NS	*
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	NS	NS	NS
oleic	**	*	NS	NS	NS
linoleic	NS	NS	NS	*	***
linolenic	NS	-[a]	NS	NS	-
eicosatri-enoic	-	NS	NS	NS	NS
arachidonic	NS	NS	-	-	NS
docosapent-aenoic	NS	NS	NS	NS	NS
docosahex-aenoic	**	*	-	-	NS

The results refer to the data shown in Tables 99, 100.
[a]- notation as in Table 89b.
Significance of difference- notations as in Table 89a.

Table 101b The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the heart microsomes between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	26.54 ±3.92	32.44 ±3.54	27.69 ±4.01	26.36 ±3.28	47.58 ±0.90	45.78 ±1.53
palmitoleic	5.85 ±1.17	5.88 ±1.99	14.76 ±5.67	6.43 ±1.78	2.76 ±1.98	4.58 ±2.84
stearic	9.66 ±1.99	15.08 ±3.78	9.68 ±1.74	11.78 ±0.62	17.83 ±0.63	17.84 ±1.17
oleic	44.17 ±2.70	42.03 ±3.89	37.01 ±0.74	32.84 ±2.40	30.71 ±2.29	25.79 ±2.69
linoleic	9.09 ±1.51	4.08 ±2.35	4.88 ±0.75	11.85 ±1.46	0.56 ±0.36	5.99 ±3.22
linolenic	ND[b]	ND	ND	4.79 ±2.64	ND	Tr [a]
eicosatri-enoic	ND	ND	Tr	0.69 ±0.13	Tr	Tr
arachidonic	2.45 ±1.24	0.45 ±0.30	1.70 ±0.74	4.14 ±0.30	Tr	Tr
docosapent-aenoic	ND	ND	ND	ND	ND	ND
docosahex-aenoic	2.24 ±0.73	Tr	ND	1.46 ±0.43	Tr	ND

Number of replicates as for Table 93.
[a,b]- notations as in Table 87.

Table 102 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the brain microsomes of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	31.30 ±2.42	NA[c]	30.72 ±0.94	36.07 ±1.75	37.42 ±0.92	23.14 ±4.41
palmitoleic	5.89 ±0.63	NA	6.06 ±1.16	5.37 ±3.27	7.74 ±0.50	11.16 ±2.48
stearic	13.47 ±2.76	NA	17.07 ±4.03	17.79 ±4.73	13.83 ±1.79	22.19 ±2.60
oleic	40.24 ±2.12	NA	37.97 ±2.44	24.06 ±1.58	34.39 ±1.01	42.46 ±4.81
linoleic	9.10 ±1.23	NA	7.69 ±0.66	5.90 ±0.35	6.62 ±0.61	1.04 ±0.21
linolenic	ND[b]	NA	ND	ND	ND	ND
eicosatri-enoic	ND	NA	ND	ND	ND	ND
arachidonic	Tr [a]	NA	Tr	0.80 ±0.48	Tr	Tr
docosapent-aenoic	ND	NA	ND	Tr	ND	ND
docosahex-aenoic	ND	NA	ND	ND	ND	ND

Number of replicates as for Table 94.
[a,b,c]- notations as in Tables 87, 88 respectively.

Table 103 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the brain microsomes of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	***	**
	(Y)[b]	NS	*	NS	*
palmitoleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
stearic	(M)	NS	NS	***	**
	(Y)	NS	NS	NS	NS
oleic	(M)	*	NS	NS	NS
	(Y)	NS	**	**	*
linoleic	(M)	*	**	***	NS
	(Y)	NS	NS	NS	NS
linolenic	(M)	NS	-[c]	-	-
	(Y)	NS	NS	NS	***
eicosatri-enoic	(M)	NS	**	**	**
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	*	***	***
	(Y)	NS	-	-	-
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	*	*	*	*
	(Y)	NS	NS	NS	NS

The results refer to the data shown in Tables 102, 103.
[a,b,c]- notations as in Table 89a.
Significance of difference- notations as in Table 89a.

Table 104a The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the brain microsomes during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	*	***	**
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	NS	NS	NS
oleic	NS	NS	*	NS	*
linoleic	NS	*	**	***	NS
linolenic	NS	NS	-[a]	NS	NS
eicosatri-enoic	NS	NS	**	NS	NS
arachidonic	-	-	**	NS	NS
docosapent-aenoic	NS	NS	NS	NS	NS
docosahex-aenoic	*	NS	*	NS	NS

The results refer to the data shown in Tables 102, 103.
[a]- notation as in Table 89a.
Significance of difference- notations as in Table 89a.

Table 104b The levels of significance for the changes in relative concentrations of the major fatty acids in the cholesteryl esters of the brain microsomes between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	5.18 ±1.50	9.40 ±3.30	3.04 ±0.28	4.26 ±0.24	6.44 ±0.71	10.30 ±2.20
palmitoleic	1.57 ±0.53	1.64 ±0.85	2.00 ±0.14	1.52 ±0.03	3.77 ±0.52	4.99 ±0.61
stearic	2.19 ±0.28	2.91 ±0.45	2.69 ±0.20	3.38 ±0.34	1.89 ±0.96	8.40 ±1.65
oleic	73.45 ±1.57	68.22 ±3.65	74.18 ±0.93	69.99 ±3.55	79.12 ±0.88	58.92 ±2.09
linoleic	11.44 ±0.96	12.85 ±0.55	14.29 ±0.68	14.78 ±0.80	7.00 ±1.28	15.21 ±3.55
linolenic	Tr [a]	0.30 ±0.09	0.56 ±0.10	0.72 ±0.04	1.42 ±0.42	0.97 ±0.77
eicosatri-enoic	Tr	Tr	Tr	0.16 ±0.02	ND[b]	ND
arachidonic	1.92 ±0.29	1.69 ±0.29	1.49 ±0.15	2.31 ±0.06	ND	Tr
docosapent-aenoic	Tr	ND	0.43 ±0.35	1.40 ±0.40	ND	ND
docosahex-aenoic	4.16 ±0.79	2.99 ±0.74	1.34 ±0.11	1.47 ±0.47	0.36 ±0.26	ND

Number of replicates as for Table 87.
[a,b]- notations as in Table 87.

Table 105 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the liver supernatant of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	5.22 ±0.29	NA[c]	3.72 ±0.19	6.62 ±2.36	4.56 ±1.95	13.12 ±1.51
palmitoleic	3.14 ±0.34	NA	1.55 ±0.10	1.50 ±0.60	2.93 ±0.87	5.76 ±0.88
stearic	2.51 ±0.26	NA	2.94 ±0.49	2.63 ±0.81	1.00 ±0.40	5.28 ±0.12
oleic	69.02 ±1.23	NA	74.33 ±0.77	73.18 ±0.30	80.27 ±5.97	62.02 ±6.08
linoleic	10.77 ±0.43	NA	13.33 ±0.48	14.22 ±0.90	9.40 ±1.92	12.71 ±4.24
linolenic	0.74 ±0.24	NA	0.54 ±0.14	0.49 ±0.17	0.38 ±0.20	1.10 ±0.24
eicosatri-enoic	ND[b]	NA	ND	ND	Tr [a]	ND
arachidonic	3.43 ±0.32	NA	1.65 ±0.10	1.90 ±0.12	Tr	ND
docosapent-aenoic	0.16 ±0.13	NA	ND	ND	ND	ND
docosahex-aenoic	5.02 ±0.43	NA	1.44 ±0.56	1.68 ±0.18	1.41 ±0.82	ND

Number of replicates as for Table 87.
[a,b,c]- notations as in Tables 87, 88 respectively.

Table 106 The relative concentrations (weight percentages ± SE each fatty acid) of the major fatty acid components in the cholesteryl esters of the liver supernatant of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	*	*	*
	(Y)[b]	**	NS	NS	NS
palmitoleic	(M)	NS	*	**	**
	(Y)	**	NS	NS	**
stearic	(M)	NS	NS	NS	*
	(Y)	NS	NS	NS	*
oleic	(M)	*	NS	*	*
	(Y)	NS	NS	NS	NS
linoleic	(M)	NS	NS	**	NS
	(Y)	**	NS	NS	NS
linolenic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
eicosatri-enoic	(M)	NS	-[c]	-	-
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	**	***	***
	(Y)	**	NS	***	***
docosapent-aenoic	(M)	-	NS	-	-
	(Y)	-	NS	NS	NS
docosahex-aenoic	(M)	NS	NS	-	-
	(Y)	**	NS	NS	***

The results refer to the data shown in Tables 105, 106.
[a,b,c]- notations as in Table 89a.
Significance of difference- notations as in Table 89a.

Table 107a The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the liver supernatant during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	NS	NS
palmitoleic	*	NS	NS	NS	NS
stearic	NS	NS	NS	NS	NS
oleic	NS	NS	NS	NS	NS
linoleic	NS	NS	NS	NS	NS
linolenic	-[a]	NS	NS	NS	NS
eicosatri-enoic	NS	NS	NS	NS	NS
arachidonic	*	NS	NS	NS	NS
docosapent-aenoic	NS	-	-	NS	NS
docosahex-aenoic	NS	NS	NS	NS	NS

The results refer to the data shown in Tables 105, 106.
[a]- notation as in Table 89b.
Significance of difference- notations as in Table 89a.

Table 107b The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the liver supernatant between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	18.15 ±1.30	12.40 ±2.69	11.48 ±1.24	12.94 ±3.07	20.53 ±0.63	15.28 ±0.80
palmitoleic	2.76 ±0.75	4.51 ±0.19	6.40 ±3.40	2.82 ±0.49	13.11 ±3.34	5.06 ±1.11
stearic	5.02 ±0.38	2.83 ±0.59	3.55 ±1.14	8.83 ±1.14	7.33 ±3.01	16.54 ±4.94
oleic	45.53 ±1.93	53.25 ±1.62	50.60 ±2.54	44.40 ±5.51	30.68 ±2.26	34.45 ±1.28
linoleic	20.92 ±1.40	21.92 ±0.40	21.24 ±2.00	24.71 ±1.76	23.44 ±0.34	26.44 ±3.31
linolenic	0.32 ±0.12	0.93 ±0.20	0.87 ±0.44	0.52 ±0.12	2.58 ±1.28	0.59 ±0.19
eicosatri-enoic	0.46 ±0.18	Tr [a]	Tr	0.58 ±0.01	0.97 ±0.37	ND[b]
arachidonic	3.87 ±0.19	2.40 ±0.04	3.91 ±0.28	3.94 ±0.38	0.48 ±0.28	0.24 ±0.14
docosapent-aenoic	Tr	0.10 ±0.01	0.21 ±0.11	0.22 ±0.02	ND	ND
docosahex-aenoic	2.94 ±0.75	1.58 ±0.33	1.60 ±0.60	1.09 ±0.19	0.88 ±0.45	1.39 ±0.82

Number of replicates as for Table 90.
[a,b]- notations as in Table 87.

Table 108 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the heart supernatant of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	16.24 ±1.68	NA[c]	12.18 ±2.48	18.14 ±3.26	14.20 ±0.78	15.04 ±2.64
palmitoleic	2.89 ±0.12	NA	2.96 ±0.04	4.54 ±1.39	8.23 ±0.86	6.48 ±1.47
stearic	5.02 ±0.62	NA	4.56 ±0.44	5.80 ±1.56	3.16 ±0.18	2.54 ±1.61
oleic	56.02 ±2.12	NA	55.22 ±3.66	52.85 ±0.30	35.75 ±1.87	32.96 ±2.20
linoleic	18.02 ±1.13	NA	19.68 ±0.07	15.80 ±2.44	34.41 ±2.16	40.66 ±2.29
linolenic	Tr [a]	NA	Tr	0.50 ±0.34	1.04 ±0.40	0.97 ±0.26
eicosatri-enoic	Tr	NA	Tr	ND[b]	Tr	0.21 ±0.06
arachidonic	1.10 ±0.13	NA	3.74 ±0.10	2.36 ±1.72	2.68 ±1.53	0.35 ±0.01
docosapent-aenoic	Tr	NA	ND	ND	ND	ND
docosahex-aenoic	Tr	NA	ND	ND	0.46 ±0.03	0.71 ±0.21

Number of replicates as for Table 91.
[a,b,c]- notations as in Tables 87, 88 respectively.

Table 109 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the heart supernatant of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	**	NS	NS	NS
	(Y)[b]	NS	NS	NS	NS
palmitoleic	(M)	NS	NS	*	NS
	(Y)	NS	NS	NS	NS
stearic	(M)	NS	*	NS	NS
	(Y)	NS	NS	NS	NS
oleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	***	***
linoleic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	**	***
linolenic	(M)	NS	NS	NS	NS
	(Y)	NS	-	NS	NS
eicosatri-enoic	(M)	-[c]	***	NS	***
	(Y)	NS	NS	NS	*
arachidonic	(M)	NS	NS	***	***
	(Y)	NS	NS	NS	NS
docosapent-aenoic	(M)	NS	NS	-	-
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	***	*

The results refer to the data shown in Tables 108, 109.
[a,b,c]- notations as in Table 89a.
Significance of difference- notations as in Table 89a.

Table 110a The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the heart supernatant during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	NS	NS	NS	***	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	NS	NS	*
oleic	*	NS	NS	NS	NS
linoleic	NS	NS	*	**	*
linolenic	-[a]	-	NS	NS	NS
eicosatri-enoic	-	NS	***	-	*
arachidonic	***	NS	NS	NS	NS
docosapent-aenoic	NS	-	***	NS	NS
docosahex-aenoic	**	NS	**	NS	NS

The results refer to the data shown in Tables 108, 109.
[a]- notation as in Table 89b.
Significance of difference- notations as in Table 89a.

Table 110b The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the heart supernatant between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	22.51 ±3.54	18.90 ±1.62	17.92 ±1.96	16.22 ±1.39	25.89 ±1.45	28.67 ±3.78
palmitoleic	4.36 ±1.44	7.60 ±2.56	7.67 ±1.60	0.68 ±0.18	10.68 ±4.79	8.30 ±0.78
stearic	4.86 ±0.76	8.35 ±2.14	5.24 ±0.85	6.10 ±0.54	11.57 ±0.19	20.06 ±4.62
oleic	46.92 ±1.69	45.76 ±0.80	48.62 ±0.01	59.57 ±0.28	42.63 ±0.28	28.12 ±3.55
linoleic	17.50 ±1.65	13.89 ±1.93	15.91 ±4.11	16.78 ±1.63	7.83 ±2.01	10.85 ±3.61
linolenic	ND[a]	0.88 ±0.58	0.93 ±0.33	0.35 ±0.15	ND	ND
eicosatri-enoic	ND	ND	ND	ND	ND	ND
arachidonic	2.78 ±1.44	4.62 ±0.64	3.69 ±0.49	0.32 ±0.12	1.26 ±0.46	ND
docosapent-aenoic	ND	ND	ND	ND	ND	ND
docosahex-aenoic	1.07 ±0.47	ND	ND	ND	ND	ND

Number of replicates as for Table 93.
[a]= no detected concentration.

Table 111 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the brain supernatant of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated			days after hatch		
	13d	16d	19d	1DO	6DO	12DO
palmitic	6.47 ±1.23	NA[c]	6.45 ±1.25	13.24 ±0.36	15.83 ±4.63	32.13 ±2.56
palmitoleic	2.13 ±0.34	NA	2.73 ±1.34	2.34 ±0.93	6.98 ±3.11	10.94 ±2.59
stearic	4.76 ±0.54	NA	3.39 ±0.03	2.64 ±1.07	6.67 ±2.65	9.84 ±0.80
oleic	67.12 ±0.81	NA	68.04 ±0.77	64.33 ±2.78	56.36 ±2.93	34.74 ±2.48
linoleic	14.82 ±0.76	NA	13.85 ±1.85	12.53 ±0.82	13.02 ±0.15	10.24 ±2.43
linolenic	0.32 ±0.12	NA	0.24 ±0.01	0.52 ±0.30	ND[b]	Tr [a]
eicosatri-enoic	ND	NA	ND	ND	ND	Tr
arachidonic	2.32 ±0.34	NA	3.01 ±1.54	3.24 ±1.01	1.14 ±0.24	1.56 ±0.40
docosapent-aenoic	ND	NA	ND	ND	ND	ND
docosahex-aenoic	2.06 ±0.44	NA	2.30 ±0.31	1.16 ±0.82	ND	ND

Number of replicates as for Table 94.
[a,b,c]- notations as in Tables 87, 88 respectively.

Table 112 The relative concentrations (weight percentages ± SE of each fatty acid) of the major fatty acid components in the cholesteryl esters of the brain supernatant of embryos and chicks from young parents.

fatty acid:		13d v 19d	19d v 1DO	1DO v 6DO	1DO v 12DO
palmitic	(M)[a]	NS	NS	**	*
	(Y)[b]	NS	**	NS	***
palmitoleic	(M)	NS	**	NS	***
	(Y)	NS	NS	NS	*
stearic	(M)	NS	NS	***	*
	(Y)	*	NS	NS	**
oleic	(M)	NS	***	***	***
	(Y)	NS	NS	NS	***
linoleic	(M)	NS	NS	*	NS
	(Y)	NS	NS	NS	NS
linolenic	(M)	-[c]	NS	-	-
	(Y)	NS	NS	-	-
eicosatri-enoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
arachidonic	(M)	NS	***	NS	-
	(Y)	NS	NS	NS	NS
docosapent-aenoic	(M)	NS	NS	NS	NS
	(Y)	NS	NS	NS	NS
docosahex-aenoic	(M)	-	NS	NS	NS
	(Y)	NS	NS	-	-

The results refer to the data shown in Tables 111, 112.
[a,b,c]- notations as in Table 89a.
Significance of difference- notations as in Table 89a.

Table 113a The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the brain supernatant during embryonic and post-hatch development.

fatty acid:	13d v 13d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO
palmitic	**	**	NS	NS	NS
palmitoleic	NS	NS	NS	NS	NS
stearic	NS	NS	*	NS	NS
oleic	***	***	NS	**	NS
linoleic	NS	NS	NS	*	NS
linolenic	-[a]	NS	NS	NS	NS
eicosatri-enoic	NS	NS	NS	NS	NS
arachidonic	NS	NS	*	NS	**
docosapent-aenoic	NS	NS	NS	NS	NS
docosahex-aenoic	NS	***	-	NS	NS

The results refer to the data shown in Tables 111, 112.
[a]- notation as in Table 89b.
Significance of difference- notations as in Table 89a.

Table 113b The levels of significance for the changes in relative concentrations of the major fatty acid components in the cholesteryl esters of the brain supernatant between embryos and chicks from mature and young parents.

fatty acid:	days after incubation initiated						days after hatch		
	13d			19d			6DO		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
palmitic	42.56	49.89	7.55	38.24	55.95	5.80	61.67	35.21	3.12
palmitoleic	26.32	73.68	ND[b]	21.95	78.05	ND	63.68	33.33	2.98
stearic	45.44	48.43	6.13	41.32	54.95	3.70	62.80	34.29	2.91
oleic	40.87	47.88	11.25	37.72	55.11	7.17	64.83	31.94	3.23
linoleic	42.80	50.19	7.01	41.45	53.10	5.45	63.27	33.88	2.88
linolenic	-[a]	-	-	-	-	-	63.47	36.27	0.26
eicosatrienoic	50.00	47.56	2.44	63.11	34.43	2.46	43.24	45.27	11.47
arachidonic	44.55	52.21	3.25	36.09	58.70	5.01	71.82	26.89	1.29
docosapentaenoic	-	-	-	-	-	-	-	-	-
docosahexaenoic	40	53.47	6.53	32.55	63.55	4.21	64.64	33.78	1.58

The number of replicates used for all analyses was- embryos 5, chicks 4.
[a]= values too low to allow accurate comparisons.
[b]= no detected concentration.

Table 114 The percentage distributions of the major fatty acids between the phospholipids of the mitochondria, microsomes and supernatant of livers of embryos and chicks from mature parents.

fatty acid:	days after incubation initiated						days after hatch		
	13d			19d			6DO		
	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant	mitochondria	microsomes	supernatant
palmitic	60.6	33.5	5.8	57.2	40.31	2.51	73.7	23.5	2.7
palmitoleic	52.8	49.4	2.8	67.6	31.97	0.40	79.8	17.5	2.6
stearic	60.9	35.8	3.3	60.7	36.70	2.58	75.7	21.9	2.3
oleic	68.0	26.8	5.2	55.8	38.50	5.65	74.4	22.7	2.9
linoleic	72.3	27.6	0.1	62.0	34.59	3.38	76.7	21.0	2.3
linolenic	52.6	31.6	15.8	-[a]	-	-	75.7	23.5	0.8
eicosatrienoic	24.4	9.10	66.5	42.8	55.72	1.53	75.5	22.0	2.5
arachidonic	67.8	32.2	0	64.2	33.94	1.90	83.2	15.1	1.6
docosapentaenoic	-	-	-	-	-	-	-	-	-
docosahexaenoic	63.1	32.1	4.8	63.4	34.79	1.81	75.9	22.1	2.0

The number of replicates for all analyses was 4.
[a]- notation as in Table 114.

Table 115 The percentage distributions of the major fatty acids between the phospholipids of the mitochondria, microsomes and supernatant of livers of embryos and chicks from young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	desmosterol	cholesterol	ratio of desmosterol/ cholesterol	desmosterol	cholesterol	ratio of desmosterol/ cholesterol
13d	0.78 ± 0.08	6.31 ± 0.34	0.12	2.06 ± 0.31	17.32 ± 2.12	0.12
16d	0.42 ± 0.16	2.97 ± 0.69	0.14	NA[c]	NA	NA
19d	0.37 ± 0.08	6.15 ± 0.68	0.06	1.86 ± 0.38	15.66 ± 2.71	0.12
days after hatch:						
1DO	0.04 ± 0.02	2.30 ± 0.49	0.02	0.48 ± 0.23	11.46 ± 3.84	0.04
6DO	0.03 ± 0.01	5.91 ± 1.00	5.1×10^{-3}	0.28 ± 0.11	21.4 ± 3.95	0.01
12DO	0.04 ± 0.02	4.60 ± 1.07	8.7×10^{-3}	0.31 ± 0.06	15.54 ± 4.50	0.02

The number of replicates used for all analyses was 4.
[a]= thirty-seven to forty weeks of age.
[b]= twenty-three to twenty-four weeks of age.
[c]= no samples available due to high embryo mortality.

Table 116a Total concentrations of desmosterol and cholesterol (mean weight in milligrams ± SE of the sterol) associated with the mitochondria per 10 grams of brain tissue from embryos and chicks of mature and young parents.

days after incubation initiated:	embryos and chicks from mature parents[a]			embryos and chicks from young parents[b]		
	desmosterol	cholesterol	ratio of desmosterol/ cholesterol	desmosterol	cholesterol	ratio of desmosterol/ cholesterol
13d	0.24 ± 0.03	2.58 ± 0.28	0.09	1.20 ± 0.24	9.21 ± 1.21	0.13
16d	0.24 ± 0.07	2.66 ± 0.79	0.09	NA[c]	NA	NA
19d	0.14 ± 0.06	2.86 ± 0.52	0.05	0.86 ± 0.16	7.87 ± 1.25	0.11
days after hatch:						
1DO	0.02 ± 0.01	1.84 ± 0.40	0.01	0.18 ± 0.06	4.90 ± 0.98	0.04
6DO	0.08 ± 0.02	0.96 ± 0.26	0.08	0	3.45 ± 0.50	0
12DO	1.18 ± 0.43	1.18 ± 0.43	0	0	3.41 ± 1.15	0

The number of replicates used for all analyses was 4.
a,b,c- notations as in Table 116a.

Table 116b Total concentrations of desmosterol and cholesterol (mean weight in milligrams ± SE of the sterol) associated with the microsomes per 10 grams of brain tissue from embryo and chicks of mature and young parents.

	embryos and chicks from mature parents		embryos and chicks from young parents	
	desmosterol	cholesterol	desmosterol	cholesterol
13d v 19d	*	NS	NS	NS
19d v 1DO	**	**	*	NS
1DO v 6DO	NS	*	NS	NS
1DO v 12DO	NS	NS	NS	NS

The results refer to the data shown in Table 116a.
Significance of difference: *=P<0.05 **=P<0.01 ***=P<0.001
NS= Not significant

Table 117a The levels of significance for the changes in desmosterol and cholesterol concentrations in the mitochondria of the brain during embryonic and post-hatch development.

	embryos and chicks from mature parents		embryos and chicks from young parents	
	desmosterol	cholesterol	desmosterol	cholesterol
13d v 19d	NS	NS	NS	NS
19d v 1DO	NS	NS	**	NS
1DO v 6DO	*	NS	-a	NS
1DO v 12DO	NS	NS	-	NS

The results refer to the data shown in Table 116b.
Significance of difference-notations as in Table 117a.

Table 117b The levels of significance for the changes in desmosterol and cholesterol concentrations in the microsomes of the brain during embryonic and post-hatch development.

	mitochondria		microsomes	
	desmosterol	cholesterol	desmosterol	cholesterol
13d v 13d	**	**	**	**
19d v 19d	**	*	**	*
1DO v 1DO	NS	NS	*	*
6DO v 6DO	NS	**	NS	**
12DO v 12DO	**	NS	-[a]	NS

The results refer to the data shown in Tables 116a and b.
Significance of difference-notations as for Table 117a.

Table 117c The levels of significance for the changes in desmosterol and cholesterol concentrations in the mitochondria and microsomes of the brain between embryos and chicks from mature and young parents.

	day 15			day 19		
	CE	TG	PL	CE	TG	PL
yolk contents:						
linoleic	24.2 ± 0.40	16.5 ± 0.72	14.0 ± 0.52	23.0 ± 0.52	16.6 ± 0.51	13.9 ± 0.60
arachidonic	1.35 ± 0.09	<1.0	3.90 ± 0.14	1.44 ± 0.04	<1.0	4.05 ± 0.20
docosahexaenoic	2.47 ± 0.35	<1.0	3.61 ± 0.16	3.21 ± 0.30	<1.0	2.61 ± 0.19
concentration of PUFA in total lipid	17.6			17.2		
yolk sac membrane:						
linoleic	11.9 ± 1.02	12.7 ± 0.79	13.4 ± 0.40	11.1 ± 0.95	15.7 ± 1.81	15.8 ± 1.31
arachidonic	1.0 ± 0.06	<1.0	6.72 ± 0.56	<1.0	<1.0	7.00 ± 0.71
docosahexaenoic	1.35 ± 0.07	<1.0	4.79 ± 0.30	<1.0	<1.0	3.84 ± 0.41
concentration of PUFA in total lipid	14.3			17.3		
liver:						
linoleic	10.6 ± 0.63	10.1 ± 1.03	13.6 ± 0.44	12.2 ± 1.07	10.8 ± 0.95	15.4 ± 0.40
arachidonic	1.15 ± 0.09	2.61 ± 0.24	24.1 ± 0.81	1.51 ± 0.11	2.78 ± 0.21	22.7 ± 0.62
docosahexaenoic	1.66 ± 0.18	14.5 ± 2.04	8.40 ± 0.65	1.31 ± 0.27	11.8 ± 0.92	10.3 ± 0.42
concentration of PUFA in total lipid	32.9			23.6		

Each result is the mean ± SE of 6 observations.
[a]= CE-cholesteryl esters TG= triglycerides PL= phospholipids [b]= PUFA- polyunsaturated fatty acids

Table 118 Concentrations of the major polyunsaturated fatty acids (molar percentage of the total fatty acids) in the yolk contents, yolk sac membrane and liver of the chick embryo at days 15 and 19 of incubation.

tissue:		days after incubation initiated					days after hatch				
		0d	7d	13d	16d	19d	1DO	6DO	12DO	21DO	42DO
yolk	(M)[a]	161.1 ±10.9	91.5 ±15.8	114.9 ±17.4	117.3 ±9.2	42.5 ±11.1	54.5[d] ±29.2	30.7[d] ±10.0			
	(Y)[b]	154.2 ±14.3	73.3 ±10.9	99.4 ±16.2	77.4 ±10.8	14.9 ±4.5	16.9[d] ±4.1	29.8[d] ±11.5			
yolk sac membrane	(M)		69.6[d] ±13.4	110.1 ±16.1	230.5 ±22.0	373.7 ±24.2	494.2 ±40.8	105.5[d] ±30.1			
	(Y)		38.6[d] ±7.9	72.2 ±11.8	191.4 ±30.6	270.5 ±31.1	304.1 ±46.2	54.2[d] ±14.1			
liver	(M)			4.6[d] ±2.3	70.5[d] ±18.3	251.4 ±27.0	735.2 ±53.7	54.4 ±7.5	12.4 ±4.0	5.1 ±3.1	13.0 ±2.8
	(Y)			7.9[d] ±3.1	96.5[d] ±17.3	259.6 ±25.5	642.7 ±115.4	37.2 ±8.0	2.0 ±0.5	2.3 ±0.9	9.7 ±1.4
heart	(M)			7.6[d] ±0.3	1.1[d] ±0.1	12.7[d] ±2.6	24.8[d] ±3.5	8.7 ±1.0	7.7 ±1.0	6.5 ±1.1	5.0 ±1.6
	(Y)			7.9[d] ±0.3	1.6[d] ±0.6	0.4[d] ±0.2	15.3[d] ±3.8	9.1 ±2.4	3.4 ±1.4	2.8 ±1.7	3.0 ±1.1
brain	(M)			2.8[d] ±0.9	4.3[d] ±0.8	4.2[d] ±0.6	1.6[d] ±0.4	0.6 ±0.4	ND	2.4 ±1.2	ND
	(Y)			ND[c]	6.3[d] ±0.4	0.2[d] ±0.1	1.0[d] ±0.5	1.0 ±0.4	0.4 ±0.2	ND	ND

[a] = results for progeny from mature parents; [b] = results for progeny from young parents; [c] = no detected concentration.

[d] = determination carried out on a minimum number of 4 pooled samples: all other results are based on a minimum of 6 individual samples.

Table 119a The concentrations of alpha tocopherol (micrograms ± SE of total alpha tocopherol) per gram of tissue from embryos and chicks of mature and young parents.

	Yolk contents		Yolk sac membrane		liver	
	Day 16	**Day 19**	**Day 16**	**Day 19**	**Day 16**	**Day 19**
No of double bonds*	33	30	41	41	87	92
alpha tocopherol	117.3	42.5	230.5	373.5	70.5	251.4

* = An estimate of the state of unsaturation in the phospholipid fraction. For each day, the proportion of the major unsaturated fatty acids ie. 18:2, 20:4 and 22:6 were multiplied by a factor based on the relative content of double bonds ie. 18:2 = 1, 20:4 = 2, 22:6 = 3

The results are based on data taken from 119a.

Table 119b An estimate of the state of unsaturation versus alpha tocopherol concentration (μg per gram of tissue) in the yolk, yolk sac membrane and liver of the chick embryo at 16 and 19 days of incubation. The results are taken from embryos of mature parents.

days after incubation initiated:		days after incubation initiated					days after hatch				
		0d	7d	13d	16d	19d	1DO	6DO	12DO	21DO	42DO
0d	(M)[a]		**	*	*	***	***	***			
	(Y)[b]		**	*	**	***	***	***			
7d	(M)			NS	NS	*	NS	*			
	(Y)			NS	NS	***	***	*			
13d	(M)				NS	**	NS	**			
	(Y)				NS	***	***	**			
16d	(M)					***	NS	***			
	(Y)					***	***	*			
19d	(M)						NS	NS			
	(Y)						NS	NS			
days after hatch:											
1DO	(M)							NS			
	(Y)							NS			
6DO	(M)										
	(Y)										
12DO	(M)										
	(Y)										
21DO	(M)										
	(Y)										

The results refer to the data shown in Table 118.

[a,b]- notations as in Table 118. Significance of difference: *=P<0.05 **=P<0.01 ***=P<0.001.

Table 120 The levels of significance for the changes in concentration of alpha tocopherol in the yolk during embryonic and post-hatch development.

days after incubation initiated:		days after incubation initiated					days after hatch				
		0d	7d	13d	16d	19d	1DO	6DO	12DO	21DO	42DO
0d	(M)[a]										
	(Y)[b]										
7d	(M)			NS	***	***	***	NS			
	(Y)			*	***	***	***	NS			
13d	(M)				**	***	***	NS			
	(Y)				**	***	***	NS			
16d	(M)					**	***	*			
	(Y)					NS	NS	**			
19d	(M)						*	***			
	(Y)						NS	***			
days after hatch:											
1DO	(M)							***			
	(Y)							***			
6DO	(M)										
	(Y)										
12DO	(M)										
	(Y)										
21DO	(M)										
	(Y)										

The results refer to the data shown in Table 118.

[a,b]- notations as in Table 118.

Significance of difference- notations as in Table 119.

Table 121 The levels of significance for the changes in concentration of alpha tocopherol in the yolk sac membrane during embryonic and post-hatch development

days after incubation initiated:		days after incubation initiated					days after hatch				
		0d	7d	13d	16d	19d	1DO	6DO	12DO	21DO	42DO
0d	(M)[a]										
	(Y)[b]										
7d	(M)										
	(Y)										
13d	(M)				**	***	***	***	NS	NS	NS
	(Y)				**	***	***	**	NS	NS	NS
16d	(M)					***	***	NS	*	**	*
	(Y)					***	**	*	***	***	**
19d	(M)						***	***	***	***	***
	(Y)						**	***	***	***	***
days after hatch:											
1DO	(M)							***	***	***	***
	(Y)							***	***	***	***
6DO	(M)								***	***	***
	(Y)								**	**	**
12DO	(M)									NS	NS
	(Y)									NS	***
21DO	(M)										NS
	(Y)										**

The results refer to the data shown in Table 118.
[a,b]- notations as in Table 118. Significance of difference- notations as in Table 119.

Table 122 The levels of significance for the changes in concentration of alpha tocopherol in the liver during embryonic and post-hatch development.

days after incubation initiated:		days after incubation initiated					days after hatch				
		0d	7d	13d	16d	19d	1DO	6DO	12DO	21DO	42DO
0d	(M)[a]										
	(Y)[b]										
7d	(M)										
	(Y)										
13d	(M)				***	NS	*	NS	NS	NS	NS
	(Y)				***	***	NS	NS	*	*	**
16d	(M)					NS	**	*	*	NS	*
	(Y)					NS	NS	*	NS	NS	NS
19d	(M)						NS	NS	NS	NS	NS
	(Y)						NS	**	NS	NS	NS
days after hatch:											
1DO	(M)							*	*	*	**
	(Y)							NS	NS	NS	NS
6DO	(M)								NS	NS	NS
	(Y)								NS	*	NS
12DO	(M)									NS	NS
	(Y)									NS	NS
21DO	(M)										-[c]
	(Y)										NS

The results refer to the data shown in Table 118.

[a,b]- notations as in Table 118. [c]=values too low to allow accurate comparisons. Significance of difference- notations as in Table 119.

Table 123 The levels of significance for the changes in concentration of alpha tocopherol in the heart during embryonic and post-hatch development

days after incubation initiated:		days after incubation initiated					days after hatch				
		0d	7d	13d	16d	19d	1DO	6DO	12DO	21DO	42DO
0d	(M)[a]										
	(Y)[b]										
7d	(M)										
	(Y)										
13d	(M)				NS	NS	NS	NS	-[c]	NS	-
	(Y)				*	-	-	-	-	NS	NS
16d	(M)					NS	NS	NS	NS	NS	NS
	(Y)					***	***	***	***	***	***
19d	(M)						NS	NS	-	NS	-
	(Y)						NS	NS	NS	NS	NS
days after hatch:											
1DO	(M)							NS	-	NS	-
	(Y)							NS	NS	-	NS
6DO	(M)								-	NS	-
	(Y)								NS	-	-
12DO	(M)									NS	NS
	(Y)									NS	NS
21DO	(M)										-
	(Y)										NS

The results refer to the data shown in Table 118.

[a,b,c]- notations as in Table 123.

Significance of difference- notations as in Table 119.

Table 124 The levels of significance for the changes in concentration of alpha tocopherol in the brain during embryonic and post-hatch development.

	0d v 0d	7d v 7d	13d v 13d	16d v 16d	19d v 19d	1DO v 1DO	6DO v 6DO	12DO v 12DO	21DO v 21DO	42DO v 42DO
tissue:										
yolk	NS	NS	NS	*	*	NS	NS			
yolk sac membrane		NS	NS	NS	*	*	NS			
liver			NS	NS	NS	NS	NS	*	NS	NS
heart			NS	NS	NS	NS	NS	NS	NS	NS
brain			-[a]	NS	NS	NS	NS	-	-	NS

The results refer to the data shown in Table 118.
[a]= values too low to allow accurate comparisons.
Significance of difference- notations as in Table 119.

Table 125 The levels of significance for the changes in relative concentrations of alpha tocopherol in various tissues between embryos and chicks from mature and young parents.

	day of incubation	days after hatch					
tissue:	19d	1DO	2DO	3DO	4DO	5DO	6DO
gall bladder	67.4 ±6.9	44.5 ±8.7	6.8 ±2.8	38.7 ±21.3	15.6 ±4.2	1.8 ±0.6	ND[a]
entire gut	5.3 ±0.7						
fore gut		5.9 ±2.9	4.1 ±2.1	14.2 ±2.2	21.4 ±4.6	16.8 ±3.6	12.0 ±2.9
mid gut		18.8 ±6.1	12.4 ±3.3	8.4 ±2.9	21.1 ±2.6	15.9 ±1.5	16.4 ±4.0
hind gut		8.4 ±2.4	13.4 ±4.4	15.0 ±4.2	14.2 ±5.5	15.6 ±0.7	20.5 ±4.9
caecae		6.1 ±2.3	12.8 ±4.4	21.3 ±2.2	10.8 ±4.0	32.8 ±6.1	7.4 ±3.2

[a]= no detected concentration.

Table 126 The concentrations of alpha tocopherol (micrograms per gram of tissue ± SE of 6 observations) in the gall bladder and intestinal tract during embryonic and post-hatch development.