demonstrate that the conformation of the peptide is sensitive to this minor thermal transition. However, the monotonic decrease in intensity of the peptide-bond c.d. bands with increasing temperature shows that the 'pre-melt' of the lipid does not affect the gross conformation of the molecule.

At temperatures above the main lipid phase-transition temperature (i.e. >23°C) the complex is unstable and precipitates both the lipid and excess glucagon from solution and the interactions described above are abolished. If the solution is recooled to below the phase-transition temperature the complex re-forms in a totally reversible manner. When 1,2-dipalmitoyl-sn-glycero-3-phosphocholine is used the same phenomena are observed but the complex formed is stable up to 40°C (the phase transition of this lipid is 41°C).

The functional aspects of this phenomenon require investigation. These include the possibilities of serum lipoprotein involvement in glucagon action and the control of target-cell sensitivity to the hormone by regulation of plasma-membrane fluidity.

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Protein-Mediated Transfer of Phosphatidylcholine to Myelin

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From experiments in vivo with radioactive precursors of phospholipids, the different components of myelin phosphatidylcholine (the phosphate, glycerol, fatty acyl and choline moieties) are replaced at significant though different rates (Horrocks et al., 1976). Since isolated myelin possesses no biosynthetic capacity, the bulk of the myelin phospholipids must be metabolized by replacement of the whole phospholipid molecule in the myelin membrane through exchange of individual phospholipid molecules, or through membrane vesicle transfer, or metabolized while the phospholipid is still present in the plasma membrane of the myelinating cell and in contact with cell cytoplasm and endoplasmic reticulum. Experiments have been carried out to examine whether myelin phosphatidylcholine can be replaced by exchange with other membrane pools of phosphatidylcholine by a process involving soluble phosphatidylcholine-exchange proteins.

Myelin was isolated from adult rat brain and bovine white matter after tissue homogenization in 0.3 M-sucrose/0.1 mM-EDTA/10 mM-Tris/HCl (pH 7.2) (Suzuki et al., 1967). Osmotically shocked myelin (Agrawal et al., 1970) was free of NADPH-cytochrome c reductase, 5'-nucleotidase, cytochrome c oxidase and acetylcholinesterase activities, but possessed high 2':3' cyclic adenosine monophosphate 3'-phosphohydrolase activity. Electron microscopy revealed numerous multilamellar membrane structures, frequently with separations between adjacent lamellae, and individual membrane fragments, some as closed vesicles.
In the study of the transfer of phosphatidyl[14C]choline to myelin, myelin (0.2–0.5 mg of protein containing 50–130 nmol of phosphatidylcholine) was incubated with microsomal fraction or mitochondria isolated from rat liver after the intraperitoneal injection of [Me-14C]choline, or with phosphatidyl[Me-14C]choline liposomes containing 0.01% cholesteryl [9,10-3H]stearate, to correct for liposomal contamination of the myelin pellet (Kamp et al., 1973). With each donor membrane, the phosphatidylcholine pool size was similar (200–300 nmol). Incubations were carried out in the presence and absence of soluble proteins in the pH 5.1 supernatant of rat liver or with partially purified phosphatidylcholine-exchange protein from rat brain (Brammer & Sheltawy, 1976). Myelin was recovered from the incubation by centrifugation at 10000g for 10 min. In incubations with microsomal fraction and mitochondria, the myelin pellet was washed by resuspending the pellet by homogenization followed by centrifugation. The washing procedure was then repeated and the twice-washed myelin pellet resuspended in 1.0 ml of water and transferred to a scintillation vial containing 10 ml of Triton/toluene scintillator; radioactivity was then determined. On the basis of recovery of NADPH-cytochrome c reductase activity, unwashed myelin contained up to 16% of the added microsomal fraction before washing. The activity was too low to be detected after twice washing the myelin pellet.

Soluble proteins in the pH 5.1 supernatant and the partially purified phosphatidylcholine-exchange protein stimulated the transfer of phosphatidyl[14C]choline to rat and bovine brain myelin, with no increase in the phospholipid content. There was no transfer at 0°C, whereas at 37°C transfer was linear with time of incubation up to about 10% of the total myelin phosphatidylcholine exchanged. Equilibrium was reached when 15–20% of the myelin phosphatidylcholine pool had exchanged. The rate of transfer with mitochondria as phosphatidylcholine donors was 50% of the rate with liposomes and the microsomal fraction, and at equilibrium less phosphatidylcholine had been exchanged, indicating that the size of the available phosphatidylcholine pool was different for the three donor membranes. In vivo 40–80% of the total myelin phosphatidylcholine is readily exchanged (Horrocks et al., 1976). The rate of transfer between liposomes and myelin is only 14% of that obtained for transfer between liposomes and mitochondria. With guinea-pig brain myelin, Brammer & Sheltawy (1976) obtained a rate of between 10 and 20%. At the concentration used, myelin had no effect on the transfer of phosphatidylcholine between liposomes and mitochondria.

To study the effect of the phospholipid content of myelin on the transfer of phosphatidylcholine, phospholipid was partially removed by treating rat and bovine brain myelin with phospholipase C (Bacillus cereus) and removing the diacylglycerol formed by lipase (Rhizopus arrhizus) hydrolysis. With myelin from both species, there is a rapidly hydrolysable pool of phospholipid (25–30% of the total myelin phospholipid). The remaining phospholipid is more slowly hydrolysed. All phospholipids were hydrolysed to similar extents. Myelin depleted of phospholipid showed an increased rate of transfer of phosphatidylcholine from liposomes and mitochondria to myelin compared with untreated and zero-time control treated myelin. The initial rate of transfer showed an inverse relationship to the amount of phospholipid in the myelin membrane. At 50% depletion, there was a 3-fold increase in the rate of transfer with rat and bovine brain myelin. At equilibrium, the total amount of phosphatidyl[14C]choline taken up by the lipid-depleted myelin was similar to that of the untreated, suggesting that the phosphatidylcholine taken up by the depleted myelin is not replacing all the phospholipid hydrolysed by the phospholipase C, but only the phosphatidylcholine pool available for exchange and also attacked by phospholipase C.

Exchange of myelin phosphatidylcholine has also been examined by incorporating [14C]choline into rat and bovine brain myelin by incubating myelin in the presence of phospholipase D, 50 mM-CaCl2 and 200 mM-[Me-14C]choline (0.05 Ci/mol). After repeated washing of the myelin together with repeated freezing and thawing to release the occluded [14C]choline, the specific radioactivity of the myelin phosphatidylcholine pool was 8–25 d.p.m./nmol (depending on the length of time of incubation with the phospholipase D). Phosphatidyl[14C]choline was released from myelin by the pH 5.1
supernatant and transferred to phosphatidylcholine liposomes. The linear rate of transfer was similar to that for the transfer of phosphatidylcholine from liposome to myelin (10 nmol/h per mg of pH 5.1-supernatant protein).

It seems reasonable to suggest that the transfer of phosphatidylcholine to myelin is controlled by the amount of phosphatidylcholine already in the myelin membrane, as well as by other phospholipids. The ability to transfer phosphatidylcholine to and from mature myelin may be one of the means of maintaining the high phospholipid content in myelin while allowing the replacement of individual phospholipid molecules as observed in vivo.


**A Micro-Electrophoretic Investigation of the Surface of Isolated Myelin**

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**Changes in the Metabolism of High-Molecular-Weight Ribonucleic Acid in Hypothalamic and Cortical Regions of the Developing Female Rat Brain**

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During the postnatal development of the rat forebrain there are distinctive changes in the metabolism of high-molecular-weight RNA which comprises mostly rRNA and mRNA (Lim, 1977). Proportionally more mRNA is synthesized in the newborn compared with adult forebrain (Berthold & Lim, 1976a). The mRNA appears to have a higher turnover rate in the newborn (Bondy & Roberts, 1968), since there is no increase in mRNA concentration throughout development as judged by the content of poly(A) (Berthold, 1975; Lim, 1977). There is also considerable transport of newly synthesized high-molecular-weight RNA into the cytoplasm, which is probably responsible for continued accumulation of rRNA in the newborn brain. This transport is restricted in the adult brain (Adams, 1966; Berthold & Lim, 1976b). We have extended our investigations on RNA metabolism in the developing brain to a comparison of these aspects in hypothalamic and cortical regions.

High-molecular-weight radioactive RNA was isolated by phenol extraction and precipitation with 2M-LiCl from brain preparations of rats injected intracranially with [3H]P[32]P. The proportion of polyadenylated RNA was determined by oligo(dT)–cellulose chromatography. The RNA was characterized by polyacrylamide-gel electrophoresis. These techniques, as well as the preparation of nuclear and cytoplasmic fractions, have been extensively described previously (Berthold & Lim, 1976a,b). The hypothalamic region was dissected out as described by McEwen & Pfaff (1970).

In both hypothalamic and cortical regions of the young brain, polyadenylated RNA formed a larger proportion of the total cellular RNA labelled at 4 h in comparison with the adult. At this period of labelling this proportion of polyadenylated RNA was the same