EFFECTS OF CONSUMPTION OF CHOLINE AND LECITHIN
ON NEUROLOGICAL AND CARDIOVASCULAR SYSTEMS

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by

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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was prepared for the Bureau of Foods, Food and Drug Administration (FDA), by John L. Wood, Ph.D. and Richard G. Allison, Ph.D., LSRO, FASEB, in accordance with the provisions of Contract No. FDA 223-79-2275.

The LSRO acknowledges the contributions of the investigators and consultants who assisted with this study. The report reflects the opinions expressed by participants in an ad hoc study group that met at the Federation on December 1 and 2, 1980. A judicious attempt has been made to incorporate the various viewpoints and opinions. The report was reviewed by these consultants; however, the listing of their names in Section VII does not imply that they endorse the study conclusions. The authors and LSRO accept responsibility for the contents of the report.

The report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to FDA by the Executive Director, FASEB.

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Kenneth D. Fisher, Ph.D.
Director
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SUMMARY

Commercial lecithin is a complex mixture of many phosphatides, principally phosphatidylcholines, phosphatidylethanolamines, and phosphatidylinositol. In the scientific literature, in contrast to commercial usage, the term "lecithin" has been used to refer both to the commercial product and to phosphatidylcholine. This has created some confusion in interpreting reported results. In this report the use of the term lecithin is restricted to mixtures of phosphatides that can be obtained from natural sources.

Lecithin is widely distributed in plants and animals and is a common constituent in foods. Its intake varies with diet selection but ordinarily amounts to 1-5 g/d. The free choline content of the diet is very low. Dietary choline is contained in the phosphatidylcholine fraction of lecithin or is derived from hydrolysis of the phosphatide. Ingested lecithin is partly hydrolyzed during digestion. Resynthesis in the intestinal mucosa results in redistribution of the fatty acid components in accordance with physiological requirements. Further hydrolysis of phosphatidylcholine, especially in the liver, provides the body with its major source of choline and biosynthesis provides the remainder.

Choline and lecithin are relatively nontoxic but acute, intermediate, and chronic cholinergic effects of large intakes of choline are of concern. Such effects from lecithin result from its choline content and are likely to be less acute but more prolonged. Possible effects on fetal development from chronic lecithin ingestion are unknown and long-term effects of intake by man have not been studied. Development of supersensitivity of dopamine receptors and disturbance of the cholinergic-dopaminergic-serotonergic balance is a concern for prolonged intakes of large amounts of lecithin.

News articles have aroused public interest in lecithin as a dietary supplement. Large quantities of commercial lecithin are available to the public. Immediate effects from high lecithin or choline intake include gastrointestinal upset, salivation, sweating, and anorexia with later onset of depression as a potential hazard. Because of the acute distress resulting from intakes of large amounts, it seems improbable that individuals will incur lasting health hazards from self-administration of supplemental dosages of lecithin.

Free choline is utilized by practically all cells for synthesis of phosphatidylcholine, sphingomyelin, plasmalogens, and acetylcholine and some is oxidized to betaine. Phospholipids are essential components of lipoproteins and membranes. Membranes may reflect the lipid content of the diet to some extent. In most cases, biosynthesis more than dietary intake determines the characteristics of membrane phospholipids.
Chronic administration of large amounts of soybean lecithin alters lipoprotein patterns of plasma, reduces chylomicron size, and lowers cholesterol content. The preponderance of evidence indicates that a diet rich in unsaturated fats and oils is more effective than polyenoic lecithin but there are some data to the contrary.

For purposes of clinical investigations, present specifications for food grade lecithin do not permit accurate assessment of the active components in a particular preparation, especially with regard to the phosphatidylcholine and linoleic acid contents. Clinical investigations on the treatment of neurological disturbances will be limited unless sufficient amounts of pure phosphatidylcholine can be supplied.

Much research has been stimulated by reports that administration of choline or lecithin can raise plasma choline and subsequently brain choline and acetylcholine levels. In addition, choline may be provided to the brain in situ by hydrolysis of phosphocholine, phosphatidylcholine, and acetylcholine. There are indications from preliminary investigations that oral choline and phosphatidylcholine might alleviate neurological motor disturbances, prevent hypercholesteremia and atherosclerosis, and restore cognition and memory. Acetylcholine levels in the brain are maintained fairly constant by a complex control system involving end-product feedback inhibition, mass action, variations in rate of transport into neurons, availability of energy sources, and rates of nerve stimulation. The neuronal choline concentration, the rate-limiting factor in acetylcholine synthesis, is normally controlled by a "high-affinity" choline transport system. Most investigators believe the choline level of the brain can be raised also by increased supply through a "low-affinity" transport system. Raising the brain choline levels has been reported to increase acetylcholine synthesis in the brain or produce a direct choline agonist effect.

Studies with aged animals indicate that increasing cholinergic function with choline or other cholinergic agonists improves performance in memory- and learning-related tests. Results of tests with normal young or elderly subjects indicate that, in man, stimulation of the cholinergic system by precursor administration has not been effective but may supplement drugs in improving memory or cognition. Results from treatment of Huntington's disease have not been encouraging because of a characteristic deficiency of receptor cholinergic neurons. Varied responses have been observed in treatment of Gilles de la Tourette's disease, Friedreich's ataxia, levodopa-induced dyskinesia, mania, and myasthenic syndrome. Symptoms of tardive dyskinesia and Alzheimer's disease have been ameliorated in some patients by administration of choline, lecithin, or phosphatidylcholine. Many of the above studies are in a preliminary stage. Investigators consider the clinical use of phosphatidylcholine in conjunction with cholinergic drugs promising.
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I. INTRODUCTION

A. SCOPE OF THE STUDY

The Bureau of Foods, Food and Drug Administration (FDA), has a continuing interest in the nutritional quality of the American diet. It is responsible for evaluating and monitoring the safety of foods, establishing regulations, and providing nutrition information to consumers. In addition, the agency encourages a regulatory climate that fosters development of foods for special dietary use and that offers protection from nutrition fraud and misleading labeling. In keeping with these responsibilities, the FDA requested that the Life Sciences Research Office (LSRO) assess the available scientific information and current expert opinion on the biological effects of dietary lecithin, phosphatidylcholine, and choline in the diet. This report is a review of the current status of development and utilization of high levels of lecithin, phosphatidylcholine, and choline as dietary supplements and the possible benefits or hazards from their consumption. The biological properties of choline and lecithin are reviewed in relation to recent suggestions that they may be useful in restoring memory and in combating neurological disturbances and hypercholesterolemia. Purity and composition of commercial lecithins, results of clinical investigations, animal studies with lecithin and choline, and the effects of lecithin on cell membrane structures are presented as pertinent. Included also are suggestions for research needed to assess the effects of chronic lecithin consumption.

The topics presented in this report were discussed by an ad hoc working group convened by the LSRO on December 1 and 2, 1980. Discussions of the ad hoc group of consultants were focused on the following topics:

- Dietary sources of cholinergic precursors
- Beneficial and adverse effects of increased consumption
  - intracellular effects
  - memory and cognition
  - age-related degenerative changes in the brain
  - adrenal and endocrine effects
  - cardiovascular effects
- Effects on the developing nervous system
- Potential adaptation phenomena
- Possible effects of withdrawal
- Research needs
B. SOURCES OF CHOLINE AND LECITHIN

The free choline content of plant and animal material is very low but may be increased by hydrolysis of choline-containing compounds during food processing (Griffith and Nyc, 1971). Lecithin in the diet is a major source of exogenous choline. With special selection, total choline in the food may amount to 500-900 mg/d (Bogert et al., 1973).

In commercial usage, the term lecithin refers to a mixture of phospholipids that can be isolated from both plant and animal tissues. Phospholipids are characterized chemically as phosphatides, 1,2-diacyl-sn-glycero-3-phosphoryl derivatives, and occur in all living cells associated with fats, oils, and sterols. Phosphatides consist of a polyhydric alcohol (usually glycerol) esterified with fatty acids and phosphoric acid (Figure 1). The phosphoric acid is further esterified with a basic nitrogenous compound such as choline, ethanolamine, or serine. An exception is phosphatidylinositol in which the base is replaced by inositol.

The principal phosphatides in commercial food grade lecithin prepared from soybeans are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol (see Table A-1, page 101). It should be noted that the term, phosphatidylcholine, as with the other phosphatides, refers to a family of compounds which vary according to the fatty acids substituted on the glycerol residue. Thus, a preparation of phosphatidylcholine, which is isolated from a natural source, will be a mixture of many molecular species. For example, Marai and Kuksis (1969) have identified over 60 molecular species of phosphatidylcholine in human plasma and erythrocytes. The fatty acids in the phosphatides composing a lecithin will differ depending upon its source. Table A-2 (page 102) shows the fatty acid distribution of a number of lecithins. The complete composition of egg yolk lecithin has been determined by Kuksis and Marai (1967) and that of soybean lecithin by Privett and Nutter (1967).

Dietary lecithin is derived from both natural sources and addition of commercial products to certain foods. Depending upon the choice of foods, intakes of lecithin from natural sources may be 1-5 g/d. The lecithins added during food processing as emulsifiers and antioxidants may provide an average per capita daily exposure of about 1.5 mg/kg body weight for adults (Select Committee on GRAS Substances, 1979). The naturally occurring lecithin and choline contents of various foods are summarized in Table 1.
FIGURE 1. Chemical structure of choline and selected phosphatides.
* R₁ and R₂ refer to fatty acid alkyl groups.
Table 1. Choline and lecithin contents of foodstuffs (mg per 100 g net weight)*

<table>
<thead>
<tr>
<th>Food</th>
<th>Choline</th>
<th>Lecithin</th>
<th>Food</th>
<th>Choline</th>
<th>Lecithin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dairy and egg products</strong></td>
<td></td>
<td></td>
<td><strong>Grains, legumes, and nuts</strong></td>
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<tr>
<td>Milk, whole</td>
<td>5.6</td>
<td>6-10</td>
<td>Wheat</td>
<td>-</td>
<td>613</td>
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<tr>
<td>Cheese</td>
<td>-</td>
<td>50-100</td>
<td>Wheat germ</td>
<td>-</td>
<td>2820</td>
</tr>
<tr>
<td>Butter</td>
<td>-</td>
<td>150</td>
<td>White flour</td>
<td>-</td>
<td>346</td>
</tr>
<tr>
<td>Egg</td>
<td>0.4</td>
<td>394</td>
<td>Polished rice</td>
<td>-</td>
<td>586</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wheat bran</td>
<td>-</td>
<td>953</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Corn meal</td>
<td>-</td>
<td>280</td>
</tr>
<tr>
<td><strong>Meats and fish</strong></td>
<td></td>
<td></td>
<td>Peanuts</td>
<td>-</td>
<td>1113</td>
</tr>
<tr>
<td>Calf liver</td>
<td>650</td>
<td>850</td>
<td>Pecans</td>
<td>-</td>
<td>333</td>
</tr>
<tr>
<td>Beef round</td>
<td>-</td>
<td>453</td>
<td>Peanut butter</td>
<td>-</td>
<td>966</td>
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<tr>
<td>Lamb chop</td>
<td>-</td>
<td>753</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamb leg</td>
<td>-</td>
<td>560</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ham</td>
<td>-</td>
<td>800</td>
<td>Vegetable</td>
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<td>Veal chop</td>
<td>-</td>
<td>646</td>
<td>Spinach</td>
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<td>Veal roast leg</td>
<td>-</td>
<td>880</td>
<td>Celery</td>
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<td>Bologna</td>
<td>-</td>
<td>400</td>
<td>Brussels sprouts</td>
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<tr>
<td>Frankfurter</td>
<td>-</td>
<td>380</td>
<td>Leeks</td>
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<td>Canadian bacon</td>
<td>-</td>
<td>533</td>
<td>Cauliflower</td>
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<tr>
<td>Trout</td>
<td>-</td>
<td>580</td>
<td>Kale</td>
<td>89</td>
<td>2</td>
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<tr>
<td>Red snapper</td>
<td>-</td>
<td>560</td>
<td>Potato (white)</td>
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<td>1</td>
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<td></td>
<td></td>
<td></td>
<td>Cabbage (white)</td>
<td>36</td>
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<tr>
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<td></td>
<td>Cabbage (savoy)</td>
<td>56</td>
<td>0.8</td>
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<td></td>
<td></td>
<td></td>
<td>Lettuce</td>
<td>16-20</td>
<td>0.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>String beans</td>
<td>21</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Carrots</td>
<td>6-13</td>
<td>5-8</td>
</tr>
</tbody>
</table>

* Modified from Wurtman, 1979.
Lecithin is available to the public in retail stores and an indeterminate number of adults supplement their diet with lecithin without clinical supervision. Intakes above 25 g/d of commercial lecithin are possible but would be self-limiting because of gastrointestinal disturbances. In clinical trials, doses of 10-100 g/d have been administered as dietary supplements to utilize the polyenoic fatty acid content for the treatment of hyperlipidemias and the phosphatidylcholine content for the treatment of neurological disturbances.

Since commercial lecithin is only 20% phosphatidylcholine, clinicians prefer to use a purified form of the latter for their clinical trials. The demand for phosphatidylcholine may stimulate commercial production of the racemic form. The unnatural enantiomer of phosphatidylcholine appears to be metabolically inert in enzyme-catalyzed reactions (Smith and Kuksis, 1980) but it may have yet undetermined toxic effects.
II. METABOLIC ASPECTS

A. DIGESTION AND ABSORPTION OF EXOGENOUS SOURCES

1. Choline

The small amount of free choline normally present in the diet can be rapidly absorbed by the intestine. However, if choline is ingested in large amounts in supplements, a large part is converted by bacteria in the intestine to trimethylamine and trimethylamine oxide (de la Huerga and Popper, 1951). Considerable amounts of these products are absorbed and excreted in sweat and urine. This not only produces an objectionable body odor, but also limits the amount of choline that can be utilized from ingestion.

2. Phosphatidylcholine and other phosphatides

Because dietary lecithin is a complex mixture of phospholipids and minor constituents, most investigations on digestion and absorption have been directed toward specific components, i.e., phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. Studies on the digestion and absorption of dietary phosphatidylcholine show that a fatty acid is removed by hydrolysis in the intestinal lumen to form lysophosphatidylcholine, which, absorbed into the intestinal mucosa, may be reacylated from the fatty acid pool (Havel, 1980; Scow et al., 1967; Thompson, 1973). Although early studies (Blomstrand, 1955; Scow et al., 1967) reported a considerable amount of phosphatidylcholine escaped digestion and was transported intact via the lymph, the estimate has more recently been lowered to 10-20% of that ingested (Hölzl and Wagner, 1971; LeKim and Betzing, 1976).

A mucosal dismutase converts two molecules of lysophosphatidylcholine to phosphatidylcholine and glycerophosphorylcholine. The latter is hydrolyzed by a phosphodiesterase to choline and glycerophosphate. There is also a lysolecithinase which converts lysophosphatidylcholine to glycerophosphorylcholine and fatty acids. The enzymes involved in the above processes have all been found in the intestinal lumens and mucosae of mammals (van den Bosch, 1974).

Dietary or luminal phosphatides are needed for the rapid clearance of large loads of dietary fat from the intestinal mucosa. Initially mixed micelles are formed composed of bile salts, phospholipids, acyl glycerols, free fatty acids, and cholesterol. LeKim and Betzing (1976) fed triply-labeled phosphatidylcholine to young rats and found its components were rapidly absorbed (as lysolecithin and fatty acids) for 6-8 h and then slowly to a total of 50% after 19 h. Radioactivity recovered from feces was only
2-5% of that fed. Half of the absorbed phosphatidylcholine was found in the lymph; half was carried as hydrolysis products, including choline, to the liver by the portal circulation. More than 90% of the labeled fatty acid moieties and half of the choline were incorporated into chylomicrons. This indicated an interchange of fatty acids on the glycerol part of the molecule accompanying triglyceride and phosphatidylcholine synthesis.

As a result of the attention given to the biological properties of phosphatidylcholine, the digestion and absorption of other phospholipid components of commercial lecithins have not been widely investigated. Phosphatidylethanolamine is apparently hydrolyzed to the lyso form. Small amounts of glycerophosphoryl-ethanolamine appear in tissues after administration of lecithin. Lysophosphatidylethanolamine may be reacylated to form a phosphatide in the intestinal mucosa. A minor part of the lyso compound can contribute to the formation of chylomicrons and very-low-density lipoproteins (VLDL) in the lymph. Water-soluble products are transported to the liver via the portal circulation.

Phosphatidylinositol may be digested and absorbed differently than phosphatidylcholine, the hydrolysis products being glycerophosphorylinositol, diglyceride, phosphate, and inositol; however, while logical, this pathway remains to be verified. Hydrolyses of inositol di- and triphosphatides are catalyzed by a phosphomonoesterase and a phosphodiesterase respectively (Holub and Kuksis, 1972). Sphingomyelin penetrates the mucosal cell and intracellular enzymes release sphingosine, fatty acids, phosphate, and choline (Nilsson, 1969).

Other aspects of dietary lecithin may affect metabolic processes. The linoleic acid moiety of lecithin provides an essential fatty acid that serves as a precursor for arachidonic acid, the substrate for prostaglandin synthesis. The phosphatidylinositol component is a source of inositol, an accessory dietary factor.

B. ENDOGENOUS SYNTHESIS

1. Choline

Endogenous synthesis of choline occurs in the endoplasmic reticulum of most tissues by transmethylation of phosphatidylethanolamine with methionine or betaine (Greenberg, 1963; Griffith and Nyc, 1971). Phosphatidylethanolamine is converted to phosphatidylcholine by stepwise transmethylation of three methyl groups from adenosylmethionine. The reaction occurs in most of the tissues but produces a large amount of phosphatidylcholine only in the liver (Björnstad and Bremer, 1966). Free choline arises from a sequence of hydrolytic steps which are common with intestinal mucosal metabolism.
The essentiality of choline in the diet has been questioned because it can be synthesized in the body via the transmethylation-phosphatidylcholine pathway. The fatty liver which is characteristic of a choline deficiency is not seen in animals maintained on an adequate diet containing methionine, betaine, or other sources of a labile methyl group. Nevertheless, Griffith and Nyc (1971) suggest that under ordinary circumstances the rate of endogenous synthesis of choline is probably inadequate to support growth of young animals or infants fed a choline-free diet.

Considerable dispute exists whether choline synthesis in adult human beings requires supplementation from the diet to meet body requirements. Dietary choline deficiency disease, resembling that developed experimentally in animals, has never been observed in man (Hartroft and Porta, 1971). This probably is a result of an ample supply of lecithin in the diet. However, Burt et al. (1980) observed abnormally low plasma choline levels in 15 patients who were maintained on total parenteral nutrition solutions that were low in methionine and contained no choline except for small amounts of phosphatidylcholine. The authors suggested that abnormal liver function associated with total parenteral nutrition may be due to choline deficiency.

At present, the relative contributions of exogenous and endogenous choline to various metabolic and biosynthetic pathways that utilize choline are unknown. Choline can be oxidized to betaine primarily in the liver and kidneys by a two-enzyme, choline oxidase system (Griffith and Nyc, 1971). Subsequent oxidation of betaine yields glycine and three formate carbons. Alternatively, betaine may transfer one of its methyl groups to homocysteine to form methionine. Choline is also phosphorylated by ATP to form phosphorylcholine, the first intermediate in synthesis of phosphatidylcholine (Kennedy and Weiss, 1956) (Figure 2) and sphingomyelin (Stoffel and Melzner, 1980). Through formation of phosphorylcholine, and subsequently phospholipids, choline is ultimately incorporated into the membranes of cells and organelles.

2. Phosphatidylcholine and other phosphatides

Three endogenous pathways lead to phosphatidylcholine and other phosphatides: (a) de novo synthesis of phosphatidylcholine and phosphatidylethanolamine from choline and ethanolamine, respectively, by the cytidine diphosphate pathway principally in the liver; (b) transmethylation of phosphatidylethanolamine; and (c) base exchange of free choline, ethanolamine, serine, or inositol with preformed phosphatides. The pathways are illustrated in Figure 2.
FIGURE 2. Schematic diagram of biosynthesis of glycerides and phosphatides.
(a) The pathways for de novo biosynthesis of phosphatides have been elucidated chiefly by Kennedy and co-workers (1961) and reviewed by McMurray and Magee, 1972; Thompson, 1973; van den Bosch, 1974; Van Golde et al., 1969. Limiting factors in synthesis of phosphatidylethanolamine and phosphatidylethanolamine are evidently the levels of choline and ethanolamine at the site of synthesis. The pathway for synthesis of phosphatidylethanolamine involves a direct reaction between cytidine diphosphodiglyceride and inositol.

(b) The transmethylases that convert phosphatidylethanolamine to phosphatidylcholine are membrane-bound. Two methyl transferases are localized in the microsomes and mitochondria of the adrenal medulla, brain, red cells, lymphocytes, mast cells, basophils, and neutrophils (Hirata and Axelrod, 1980). The enzyme that acts on phosphatidylethanolamine occurs on the inner half of the plasma membrane where the phosphatide predominates; the second enzyme occurs on the outer half of the layer, the phosphatidylcholine-rich layer.

(c) Free serine, ethanolamine, and choline exchange with the base of a phospholipid to produce phosphatidylserine, -ethanolamine, and -choline respectively (Porcellatet al., 1971). Base exchange, presumably enzymatic, is the only known pathway for synthesis of phosphatidylserine in mammalian systems (Kanfer, 1972). The incorporation of choline and ethanolamine by base exchange is of minor importance according to Sundler and Aekesson (1975) and Bjerve (1973).

Because of the variety of their metabolic interactions, phospholipids must be considered as labile components of cells (Coleman, 1973). Variations in plasma levels of phospholipids and their components may modify cellular structure and function.

Methylation and exchange of intact phospholipids in membranes with plasma phospholipids alters the distribution of phospholipids in cells and lipoproteins (McMurray, 1973). Zilversmit and coworkers (Bloj and Zilversmit, 1977; McMurray, 1973) have characterized small molecular weight proteins which accelerate the transfer of labeled phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, sphingomyelin, and cholesterol from liposomes to mitochondria or erythrocyte ghosts. The extent of the phospholipid exchange reaction in vivo and the influence of dietary lecithin are not yet clear. Hirata and Axelrod (1980) have proposed that the regulation of the phosphatidylcholine-phosphatidylethanolamine ratio affects membrane fluidity. This concept has been challenged on theoretical grounds by Vance and de Kruijff (1980).
Phospholipids are tailored to body requirements by a variety of interconversions which can exchange the fatty acids as well as the nitrogen bases. There is considerable exchange of the fatty acid residues of phospholipids by deacylation-reacylation reactions in the digestion-absorption-transport processes of the small intestine. The acyltransferases are present in many tissues and exhibit marked positional and substrate specificity (Holub and Kuksis, 1978).

The phosphatides in lecithin are important components of lipoproteins (Hamilton, 1978; Havel, 1980) and of all membrane structures; they are emulsifying agents involved in chylomicron formation; they have surfactant properties in the lungs; and they are essential to the electron transport system. Blood coagulation factor 3 of platelets is a phospholipid. Phosphatidylcholine donates the 2-acyl group for cholesterol esterification.

Phospholipids are found in the blood in the form of lipoprotein complexes (Hamilton, 1978; Havel, 1980). Lipoproteins are generated in the liver or intestine by preliminary synthesis of apoprotein-phospholipid complexes and accretion of triglycerides and cholesterol. The liver evidently catabolizes the apoproteins although there may be a recirculation of the apoprotein portion of high-density lipoproteins (HDL). Phospholipids in cells and membranes may undergo a series of alterations ranging from peroxidation of double bonds, saturation or desaturation of fatty acid moieties, redistribution of fatty acyl groups, exchange or methylation of nitrogen bases, or exchange of one phospholipid for another. This provides for rapid changes in the physical properties of phosphatides and hence of the membranes of which they are components—processes which may reflect homeostasis or adjustment to extremes of dietary intake.

C. PARENTERAL TRANSPORT

Both exogenous and endogenous choline are transported to the liver by the portal circulation. The liver metabolizes choline and releases phosphatidylcholine and very small amounts of choline to the plasma. The fasting level of plasma choline in man has been found to vary from 7–22 µM (Aquilonius and Eckernäs, 1975; Etienne et al., 1978a; Gelenberg et al., 1979a; Zeisel et al., 1980a).

The low concentration of choline in the blood and tissues, as compared to phosphatidylcholine, suggests that the latter is the chief agent of its transport and storage under ordinary dietary conditions (Björnstad and Bremer, 1966). No exclusive storage form of phospholipids has been identified but all membranes contain large amounts. The rapid turnover of phospholipids in liver, brain, and peripheral tissues (Dewailly et al., 1976) suggests that lipoproteins and membrane phosphatidylcholines supply choline by hydrolysis or base exchange.
D. CHOLINERGIC FUNCTIONS

1. Brain choline

The choline content of the brain derives from hydrolysis of acetylcholine, phosphorylcholine, membrane phosphatidylcholine and sphingomyelin, and from choline taken up from the plasma (Freeman and Jenden, 1976). There appears to be no substantial involvement of base exchange in the supply of brain choline but there is evidence for a small amount of de novo synthesis via methylation of phosphatidylethanolamine.

Much of the knowledge on the levels of choline in the brain has been obtained from studies with animals, particularly rodents. Until recently, reported values of free choline in the rodent brain have lacked agreement because of rapid postmortem autolysis of the tissues (Jope and Jenden, 1979). By killing animals with a short burst of microwave irradiation to the head, Stavinoha and Weintraub (1974) found the free choline content of rat brain to be $26.3 \pm 1.3$ nmols/g. Levels of free choline in the brain and other tissues in vivo are limited by oxidation to betaine in the mitochondria (Haubrich et al., 1981) or by phosphorylation to phosphorylcholine in the cytosol (Jope and Jenden, 1979). The phosphorylcholine content of rat brain is high (310-380 nmol/g) and may constitute a significant reservoir of choline.

Although there have been reports that choline can be synthesized in the brain de novo from ethanolamine (Kewitz and Pleul, 1976) and from deanol (dimethylaminoethanol) (Pfeiffer et al., 1957) there is presently disagreement over the claims (Freeman and Jenden, 1976; Goldberg; 1977; Zahniser et al., 1977). However, Haubrich et al. (1981) reported deanol increased free choline in kidney and liver of mice. Deanol is phosphorylated and converted to phosphatidyl(dimethylaminoethanol) which may be methylated in the liver but not in the brain (Ansell and Spanner, 1971). Alternatively the effect of deanol on choline levels in tissues could be due to base exchange with phosphatidylcholine or by inhibition of the choline oxidase system. In a review of the question, Goldberg (1977) noted that deanol administration to rats and mice causes elevation of brain acetylcholine and choline, which probably explains its beneficial effects in cholinergic therapy.

The synthesis of choline in the brain via the methylation of phosphatidylethanolamine to phosphatidylcholine has also been disputed (Ansell and Spanner, 1971). Subsequently, Blusztajn et al. (1979) and Crews et al. (1980) have detected methylation activity in brain synaptosomes from bovine and rat brains, respectively, and Blusztajn and Wurtman (1981) have observed release of free choline from the product of the methylation reaction. It is probable that phosphatidylcholine can be synthesized in the brain de novo although at a low rate (Ansell and Spanner, 1978). Ansell and Spanner (1979) have summarized evidence that choline enters
the brain in lipid-bound form, probably as phosphatidylcholine or lysophosphatidylcholine, which has been transported as lipoprotein. Pardridge et al. (1979) argued that transport of phosphatides through the blood-brain barrier is limited because both compounds are firmly bound to plasma albumin. Nevertheless, administration of labeled lysophosphatidylcholine to monkeys produced measurable radioactivity in brain tissue (Illingworth and Portman, 1972). The lyso derivative is probably transported more efficiently than the phosphatidylcholine. Illingworth and Portman (1973) have demonstrated that lysophosphatidylcholine is broken down in the brains of monkeys to produce free choline much faster than it is acylated to phosphatidylcholine.

2. Blood-brain barrier transport

The transport of free choline through the blood-brain barrier is rapid and bidirectional, but the mechanisms by which choline enters the brain from the plasma are still unclear. With normal plasma levels there is a net efflux at a rate which has been estimated to be only 5% of the rate of acetylcholine synthesis (Dross and Kewitz, 1972). There may be an influx when plasma choline levels are elevated. According to Ansell and Spanner (1979), the free choline of the blood ordinarily is not a major source of brain choline; hence choline must be generated continuously by hydrolysis of phosphatidylcholine, phosphorylcholine, or acetylcholine (Choi et al., 1975). Choline is released from acetylcholine by the action of acetylcholinesterase. This provides a renewable source of brain choline for reacetylation.

3. Neuronal Transport

Subsequent to its passage through the blood-brain barrier, choline may be transported into the neurons for conversion to acetylcholine, or it may be returned to the blood (Barker et al., 1978; Kuhar, 1978). Two transport systems for choline have been characterized from studies with brain minces and synaptosomes: a unique, high-efficiency, sodium-dependent, high-affinity system (Km, 1-10 μM); and a low-efficiency, low-affinity system (Km, 50-100 μM) (Haga and Noda, 1973; Yamamura and Snyder, 1973). The high-affinity system is also chloride-dependent (Kuhar and Zarbin, 1978) and energy-dependent (Simon and Kuhar, 1976; Yamamura and Snyder, 1973). The historical background of high-affinity uptake concept has been reviewed by Speth and Yamamura (1979). The system is probably functionally coupled to choline acetyltransferase (Barker et al., 1978; Kuhar, 1978). It may be regulated by an ion flux associated with nerve stimulation or by endogenous acetylcholine levels (Jope et al., 1978; Marchbanks and Wonnacott, 1979). Marchbanks and Wonnacott (1979) have argued against the existence of a high-affinity transport system and particularly toward coupling of transport to choline acetyltransferase activity. According to their findings on
incorporation of labeled choline into acetylcholine by guinea pig cerebral cortex synaptosomes, the transport of choline is mediated by a membrane-bound carrier with trans-activation character, i.e., it affects choline uptake opposite to the direction of transport. The choline carrier has been separated from the synaptic plasma membrane of guinea pig cerebral cortex and reincorporated into unilamellar liposomes prepared from a mixture of purified phospholipids and cholesterol approximating their distribution in synaptic plasma membranes (King and Marchbanks, 1980). The reconstituted liposomes retained properties of saturability of choline transport, sensitivity to hemicholinium-3 inhibition and membrane trans-activation.

The low-affinity system is a type shared by all cells (Diamond and Milfay, 1972). The rate of transport reflects brain choline concentrations; when the plasma level is raised by administration of choline or lecithin, there is a transient reversal of the normal efflux of choline from the brain. With continuous elevation of plasma choline a new brain level is established. Carroll and Goldberg (1975) found the high-affinity uptake system, as measured by the synthesis of acetylcholine in cortical minces, was obliterated by 35 mM K⁺, but the low-affinity system continued to function normally. Goldberg and Wecker (1980) concluded the velocity of the high-affinity transport may be insufficient to support the demands imposed by K⁺-induced depolarization. It is unlikely that the two systems operate in a cell at the same time. The low-affinity system may be located remotely and, at times of high demand, supplement the high-affinity system. Haubrich et al. (1974) and Cohen and Wurtman (1975) suggested that there is no practical limit on the rate of uptake of choline by the brain but rapid metabolism limits the levels obtained.

Regardless of the conflicting interpretations of the processes, it is evident that transport of choline consists of two distinct systems which relate to the synthesis of acetylcholine. While choline is essential for synthesis of acetylcholine, it is likely that only a small portion of exogenous choline is utilized jointly with choline generated by action of cholinesterases on acetylcholine for biosynthesis of synaptically releasable acetylcholine. Quantitation is confounded by the fact that cellular acetylcholine itself need not serve as synaptic bioamine that is released from the nerve terminal. Acetylcholine appears in non-cholinergic neurons (Karczmar et al., 1980; Koelle, 1963) and in non-nervous tissues such as placenta (Karczmar, 1967; Koelle, 1963); this acetylcholine subserves structural and permeability aspects of cell function rather than synaptic activities of cholinergic neurons. Thus, even that portion of exogenous choline that contributes to the formation of acetylcholine may not generate synaptic acetylcholine. The factors regulating acetylcholine levels in nerve tissues are reviewed on pages 17-18.
4. Acetylcholine synthesis

Acetylcholine is synthesized by the reaction of choline with acetyl coenzyme A catalyzed by choline acetyltransferase (Haubrich, 1976). The enzyme occurs widely in the body and is found in large amount in the cytoplasm of cholinergic nerve terminals. The reaction is near an equilibrium state and is thus freely reversible. However, the acetylcholine substrate for the reverse reaction is largely sequestered in bound form (Barker, 1976).

The availability of choline to choline acetyltransferase is the rate-limiting factor in regulation of acetylcholine synthesis (Barker and Mittag, 1975). This in turn is affected by the rate of transfer of choline and choline precursors through the blood-brain barrier, the release of choline from phospholipids, and by the rate of transport of choline into the neuron. There is a regional distribution of high-affinity uptake in brain that corresponds to choline acetyltransferase activity and acetylcholine concentration (Yamamura and Snyder, 1973). The highest concentration of acetylcholine is found in the corpus striatum and interpeduncular nucleus. There is little evidence of high-affinity uptake in the cerebellum. The high-affinity system probably approaches saturation at normal brain levels of choline and thus maintains a steady state of choline availability in appropriate neurons (Yamamura and Snyder, 1973). Kessler and Marchbanks (1979) concluded, however, from studies involving pulse labeling synthesis of acetylcholine by cerebral cortex synaptosomes from guinea pigs, that there did not seem to be any coupling of transport to synthesis. They interpreted their data as indicating that high-affinity uptake could only make a minor contribution to acetylcholine synthesis in cholinergic synaptosomes.

Barker (1976) has summarized current concepts on the compartmentation of acetylcholine in mammalian brain as developed by Whittaker and coworkers. There are three forms of acetylcholine experimentally demonstrable: (a) free acetylcholine in cell body and disrupted cholinergic nerve terminal cytoplasm; (b) osmotically resistant, "stable-bound", present in synaptic vesicles; and (c) osmotically labile, "labile-bound", in nerve ending cytoplasm.

5. Regulation of brain acetylcholine levels

Although increased choline plasma concentration may elevate levels of choline and acetylcholine in tissues, factors regulating acetylcholine synthesis and release are not completely understood (see pages 21-24). The promoting effects of choline on acetylcholine synthesis and release differ with the parts of the brain (Eckernäs et al., 1977; Wecker and Dettbarn, 1979). The concentration of acetylcholine in cholinergic neurons is
relatively constant under normal physiological conditions apparently because a number of factors adjust the rate of synthesis (Haubrich and Chippendale, 1977). These factors have been variously proposed as including:

(a) **Feedback inhibition of choline acetyltransferase by its endproduct, acetylcholine** (Kaita and Goldberg, 1969).

(b) **Mass action regulation of choline acetyltransferase.** Glover and Potter (1971) regarded the mass effect of substrate and product as regulatory in acetylcholine synthesis since choline acetyltransferase is reversible.

(c) **Availability of acetyl coenzyme A.** Except in times of energy deficit, the supply of acetyl coenzyme A is in excess. Jope et al. (1978) found that 50-60% of choline transported into rat brain synaptosomes by the high-affinity system was converted to acetylcholine. When the availability of acetyl coenzyme A was reduced by replacement of glucose by succinate or by inhibition of respiration by NaCN or bromopyruvate, synthesis of acetylcholine was reduced.

(d) **Changes in high- and low-affinity uptake with variations in choline levels in plasma and cholinergic neurons.** Kuhar et al. (1972) produced lesions in the medial septal nucleus which destroyed cholinergic terminals in the hippocampus. This reduced the amount of high-affinity transport and thus choline acetyltransferase activity and acetylcholine formation. There was only a slight diminution of the low-affinity uptake in the presence of 100 μM choline. Simon and Kuhar (1975) used hippocampal synaptosomes to show an impulse-flow regulation of high-affinity choline uptake in nerve terminals. When the activity of cholinergic neurons was changed by various drugs, parallel changes occurred in choline uptake. Hemicholinium-3, a highly specific inhibitor of the high-affinity transport system of the nerve terminals, can totally prevent synthesis of acetylcholine in rat synaptosomes (Guyenet et al., 1973; Haga and Noda, 1973), but uptake is reversed by additional choline (MacIntosh et al., 1956). Sherman et al. (1978) found that drugs which increased acetylcholine release increased hippocampal synaptosome uptake of choline 150% while uptake into striatal tissue was only 31%. The data on drug effects are consistent with changes in the high-affinity uptake constant produced by alterations in the cell membranes. On the other hand, Marchbanks and Wonnacott (1979) concluded that the facilitated transport of choline and hence synthesis of acetylcholine was influenced by **trans-inhibition of the membrane-bound carrier by changes of intrasynaptosomal and cytoplasmic choline and acetylcholine concentrations rather than by a high-affinity transport system.**
(e) Rate of nerve stimulation. The rapid depolarization of nerve endings with high-demand release of acetylcholine stimulates subsequent uptake of choline (Carroll and Goldberg, 1975; Mulder et al., 1974; Simon and Kuhar, 1975). K⁺-induced depolarization in mouse cortex minces showed that the capacity of the high-affinity transport system was exceeded; the low-affinity system supplied choline for immediate synthesis of acetylcholine which was not stored but released as free, active neurotransmitter. Mulder et al. (1974), however, concluded that release of acetylcholine by K⁺ depolarization was dependent on the high-affinity system. Hippocampal slices of rat brains were depolarized by suspension in 40 mM K⁺ buffer. No change in rate of choline loss to the medium was observed but acetylcholine in the tissue was 500% of the basal medium. Sherman et al. (1978) observed that depolarization by KCl was followed by a large increase in the accumulation of choline by synaptosomes from the hippocampus but there was only a slight accumulation by striatal synaptosomes. Thus, acetylcholine synthesis may be regulated differently in the two brain regions.
III. SUPPLEMENTAL INTAKES OF CHOLINE AND LECITHIN

A. INFLUENCE ON GROWTH AND DEVELOPMENT

1. Choline

Although there is an extensive literature on the effects of choline deficiencies on the growth and development of animals, little research has been done with doses known to be in excess of nutritional needs. In early studies, the growth rate of rats was depressed 20% by 2.7% dietary choline chloride, 45% by 5%, and 100% by 10% (Hodge, 1945). Higgins et al. (1945) added 160 mg/d choline chloride to the drinking water of young rats and found that within 3 wk the animals had developed a reddish brown hair pigmentation. Dogs developed a macrocytic, hyperchromic anemia after administration of 10 mg/kg choline chloride by stomach tube for 25 d (Davis, 1944). A recent review on choline functions has been made by Kuksis and Mookerjea (1978).

Participants in the LSRO ad hoc group meeting agreed that active placental transport probably protects the fetus from altered choline levels, but noted that confirming data are fragmentary.

2. Lecithin

Dietary lecithin has been generally regarded as safe and few studies on specific health effects have been made (Select Committee on GRAS Substances, 1979). No significant differences in mortality, body weight, or blood components were seen between rats fed a diet providing a mean daily intake of 1400 mg/kg body weight of soybean lecithin for 2 yr and the control animals (Brantom et al., 1973). Davis (1944) fed groups of four dogs diets providing either choline intakes of 8 mg/kg body weight/d or 5 g/d of soybean lecithin and, in both cases, observed a decrease in erythrocyte count of 15-20% within 20 d. However, the anemia disappeared after the lecithin feeding was discontinued. These observations, which were made 37 yr ago, have not been repeated. Szepesnovol (1969) observed brain tumors in mice fed lecithin at a level of 250-2500 mg/kg body weight/d or cholesterol at 200-250 mg/kg/d over their life spans. This finding has never been confirmed; no carcinogenic activity was observed by Brantom et al. (1973) in rats fed 4% soybean lecithin for 2 yr.

B. INFLUENCE ON THE FETUS AND NEWBORN

Lecithin was not teratogenic in mice at dietary levels of 16 or 1600 mg/kg body weight/d (Food and Drug Research Laboratories, Inc., 1973). Neither lecithin nor choline was mutagenic by the Ames test (Litton Bionetics, Inc., 1975; National Toxicology Program, 1981).
Whether an abnormally high choline or lecithin plasma level in the dam or fetus will alter the course of fetal or neonatal development is unknown. Zahniser et al. (1978) fed a diet supplemented with 0.8% choline to pregnant rats 15 d before delivery and 15 d after. There was no elevation of brain choline or acetylcholine in the pups. The plasma concentrations of choline were elevated in newborn infants, rats, and rabbits (Zeisel and Wurtman, 1981; Zeisel et al., 1980b). Human neonates had plasma choline levels of 39.8 μM ± 22.4 compared with 12.2 μM ± 0.3 for fasting adults. The high levels observed in infants were comparable to those known to produce pharmacological effects in adults with tardive dyskinesia (Gelenberg et al., 1979b; Growdon et al., 1977a). The significance of this finding awaits further investigation.

Welsch (1976) found that fragments of villous tissues of human placenta accumulated labeled choline. Transfer occurred by active, ATP-dependent, and simple diffusion processes. After 5 min incubation with 5 μM choline, 5% of the label was lipid-soluble and was probably in phosphatidylcholine. Of the remaining labeled material, 48% was unchanged choline, 31% acetylcholine, 5% phosphorylcholine, and 1% betaine. After 20 min, 60% of the label was found in acetylcholine. The function of placental acetylcholine is not clear.

In many mammalian species the concentration of phosphatidylcholine in the nerve tissue increases dramatically during the last stages of fetal development. At parturition the nervous system is incomplete; it continues to develop in neonatal life. Wells and Dittmer (1967), in a comprehensive study of postnatal changes in lipids of developing rat brain, found that sphingomyelin, triphosphoinositide, phosphatidic acid, galactosyldiglyceride, and inositol plasmalogens occurred in 3-d-old rats at concentrations less than 10% of those of the adult brain but then rapidly increased with the onset of myelination. Ethanolamine plasmalogen, ethanolamine phosphoglyceryl ether, cholesterol, diphosphoglycerol, and phosphatidylserine levels were 17-34% of adult brains and increased rapidly during myelination. Phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol were found at concentrations 49-60% of adult brain prior to myelination and increased only moderately during subsequent development.

There is an increased synthesis of phospholipids during late prenatal and postnatal development of rat liver (Weinhold, 1969), brain (Baker, 1970; Ogino et al., 1979), and lung (Mancalco et al., 1978; Okano and Akino, 1978). Similar changes have been observed in mice, rabbits, rhesus monkeys, and lambs (Brumley, 1971) and in human kidney (Nikolasev, 1979). An alteration in the lecithin-sphingomyelin ratio in amniotic fluid may relate to occurrence of the neonatal respiratory distress syndrome (Gluck et al., 1974). Dilinoleoyl lecithin has been shown to sensitize the rabbit uterus to oxytocin, permitting premature precipitation of labor (Lanman et al., 1972).
The role of phospholipids in maintaining the permeability and functions of cells and organelles suggests the possibility of an adverse effect from high intakes of lecithin by the gestating female. The question whether phosphatidylcholine passes the placenta has not been resolved (von Klitzing et al., 1977).

C. ALTERATIONS IN TISSUE LEVELS AND STORES

1. Plasma and other tissues

The choline contents of mammalian plasma and tissues may rise with intake but ordinarily are maintained at low levels by intestinal metabolism, oxidation, phosphorylation, and incorporation into phosphatidylcholine and sphingomyelin (Haubrich et al., 1981). The participants in the LSRO review had observed that, in their investigations, previous intake affected the fasting level and significantly influenced the increase in plasma levels that can be produced by administration of choline in the diet.

The concentration of choline in erythrocytes was found to be $14.27 \pm 2.12$ nmol/ml in 23 control subjects after a light breakfast (Hänin et al., 1980). The erythrocyte choline levels were independent of plasma choline values ($7.96 \pm 0.38$ nmol/ml).

After i.v. administration of 200 μmol/kg (1.4 mg) of choline to guinea pigs, Haubrich et al. (1974) found increases in the choline concentration of the tissues as follows: kidneys, eightfold; adrenals, threefold; heart, twofold; lungs, twofold; and liver, threefold. Acetylcholine increased fourfold in kidneys and adrenals with smaller but statistically significant increases appearing in heart, lungs, and liver. At this dose level there was no change in the brain content of either choline or acetylcholine, although changes in brain acetylcholine were observed in a later study (Haubrich et al., 1975a). In a subsequent investigation, Haubrich et al. (1975b) administered i.v. a tracer dose of $^3$H-choline to guinea pigs; the concentration of endogenous free choline after 3 min varied from 344 nmol/g in adrenal glands to 40 nmol/g in the heart and 17 nmol/g in the kidneys. There was a rapid synthesis of acetylcholine in all tissues, which did not correlate with the intracellular concentration of choline. Haubrich et al. (1976) found the metabolism of i.v. choline was accelerated in peripheral tissues of choline-deficient rats.

Choline added to the diet of rats caused sequential, dose-related increases in serum choline, brain choline, and brain acetylcholine (Cohen and Wurtman, 1976). Similar increases were found in the adrenal medulla (Ulus et al., 1977a). Choline administered i.v. rapidly elevated both choline and acetylcholine levels in the adrenals, heart, kidneys, lungs, and liver of the guinea pig (Haubrich et al., 1974). Haubrich et al. (1976) showed that dietary choline administration to rats increased the plasma level to a plateau.
Similarly, administration of lecithin equivalent to 220 mg/kg of choline in a single meal for 2 h or 400 mg/kg/d for 3 d to rats produced an increase after 10 h in the choline content of the serum of approximately 62%, in the brain of 49-72%, and in the adrenals of 60-100%. Brain acetylcholine increased 19% (Hirsch and Wurtman, 1978).

Hirsch et al. (1978) fed ten fasted subjects (18-30 yr) a single, low choline meal which was supplemented with 3 g of choline chloride. Blood samples were drawn at intervals up to 12 h after the meal. Sera were analyzed for choline, glucose, insulin, cortisol, and prolactin. Serum choline rose 86% 30 min after the test meal and approached control levels by 4 h, but none of the other substances measured was affected. The same result was obtained with a meal supplemented with 100 g of lecithin (10-20% phosphatidylcholine), except for serum choline which rose 265% in 12 h.

Elevated plasma choline levels in normal subjects can be achieved by special selection of a diet of common foodstuffs (Zeisel et al., 1980a). A diet providing only 100 mg/d choline (equivalent to 1 g lecithin) did not increase the plasma choline content above the fasting level; a diet high in choline (650 mg choline or 5.9 g of naturally-occurring phosphatidylcholine) roughly doubled the plasma level within 3-7 h after the first meal. The low choline diet supplemented with 25 g of 80% phosphatidylcholine in a coffee-flavored milkshake increased plasma choline levels 400% within 6 h and maintained an elevated level for 12 h. These and companion studies with rats showed that soybean or egg were equally effective sources of phosphatidylcholine in elevating blood choline, serum choline, or brain acetylcholine (Magil et al., 1981). Evidently the higher proportion of saturated fatty acid moieties in the egg phosphatide did not affect release of choline.

2. Brain acetylcholine

Brain acetylcholine levels are known to be sensitive to plasma levels of choline. Other tissue levels of acetylcholine are reduced by a choline deficiency also (Nagler et al., 1968). Weanling rats deprived of choline for 5 d suffered a decrease in acetylcholine of 30-35% in brain and intestine and 50-75% in kidney. Kunstscherova (1972) showed the acetylcholine content of brains, gastrointestinal tracts, and atra of starved rats was much lower than in starved rats fed a single meal containing choline. Administration of choline raised the acetylcholine tissue contents of fed rats to 130% of the controls. As noted previously, plasma choline levels and subsequently brain choline levels can be increased by dietary intake of choline esters such as the phosphatidylcholine contained in lecithin.
The principal objective of recent investigations on feeding choline and lecithin has been to increase brain acetylcholine to enhance cholinergic effects. In recent studies (Kindel and Karczmar, 1981) a dose of 120 mg/kg i.p. of choline doubled the levels of choline in the brains of mice. Increase of acetylcholine was less well-established. Although a number of early investigators failed to find increased brain acetylcholine after choline administration, the preponderance of evidence both from animal and clinical studies shows that exogenous choline (or lecithin) does increase brain acetylcholine levels. Jenden (1979) has summarized 14 reports in which parenteral administration of choline to rodents produced a rise in acetylcholine levels in brain. Five studies were noted in which no change was observed.

In initial studies, Cohen and Wurtman (1975) gave choline chloride i.p. to rats. Twenty minutes after injection of 60 mg/kg of choline chloride, brain choline levels rose to 223% of controls and then declined to base levels at 60 min. The increase in brain acetylcholine after 40 min was dose-dependent between 15 and 60 mg/kg body weight. The 40-min value after administration of 60 mg/kg was 122% of control values and returned to base levels after 80 min. Larger doses did not cause higher levels. Increased values for acetylcholine in rat brain were found also by Haubrich et al. (1975a) after i.v. or i.p. injection of choline chloride.

Cohen and Wurtman (1976) concluded that the amount of acetylcholine stored in terminals of central cholinergic neurons may vary and may reflect the choline level in the brain. They fed rats a low choline diet supplemented with 20 or 129 mg of choline daily. After 11 d the acetylcholine concentration in the caudate nucleus was 28% greater than control levels in rats consuming 20 mg/d and 45% greater in rats consuming 129 mg/d. Significant, but less marked, effects were noted in other brain structures. The acetylcholine content of cerebrum and caudate nucleus was increased greatly also by physostigmine, an inhibitor of acetylcholinesterase, given in conjunction with choline. This provided evidence for choline generation of de novo acetylcholine synthesis.

Less well-known is the effect of lecithin on choline and, particularly, acetylcholine levels in man. It is of interest that the results of the few studies performed indicate that large doses (such as 30 g/d) of lecithin or phosphatidylcholine increase markedly (sometimes three or four times) the levels of plasma choline; the effect is shortlived but may be maintained by repeated dosing (Hirsch et al., 1978; Zeisel et al., 1980). Similarly, the effects of exogenous choline or lecithin on acetylcholine turnover remain insufficiently studied. Doses of choline that cause increases of brain levels of choline and/or acetylcholine do not augment brain acetylcholine turnover; they may decrease it (Eckernäs et al., 1977; Kindel and Karczmar, 1981).
Although the increase in brain levels of acetylcholine under choline loading is not large compared with the effect on the adrenal gland (Haubrich et al., 1974, 1975b; Nagler et al., 1968; Ulus et al., 1977b), it is apparent that an increase of acetylcholine of less than twofold is significant. It was sufficient, for example, to stimulate the central cholinergic neurons to effect an increase in the metabolism of dopamine to homovanillic acid (Haubrich et al., 1979).

3. Lipoproteins and membranes

The plasma concentrations of phospholipids vary widely with fluctuations in the lipid intake. Most of the phospholipids of the plasma are incorporated into the lipoproteins. Intakes of triglycerides produce large transient increases in chylomicrons and VLDL.

Phospholipids also are found in the plasma complexed with albumin. In the fasting state, per 100 ml of plasma, the total phospholipid is 150–250 mg with phosphatidylcholine representing the major fraction (80–200 mg), lysophosphatidylcholine (15 mg), phosphatidylethanolamine (0–2 mg), plasmalogens (7–8 mg), and sphingomyelin (43–80 mg) making up the rest (Altman and Dittmer, 1974).

Virtually all organs and tissues have some capacity for formation of phospholipids from their components although rates may vary greatly. De novo synthesis, transmethylation, and phosphorylation of phospholipids are not particularly sensitive to an exogenous supply of precursors (van den Bosch, 1974).

Hawthorne (1973) has reviewed the importance of phospholipid content of nerve tissue in neurotransmission. Phosphatidylcholine and phosphatic acid take part in the process of ganglionic transmission. Polyphosphophatidylinositols are implicated in functions of the neuronal plasma membrane and are important constituents of myelin. Phospholipids are needed for activity of the sodium ATPase system.

Phospholipids are major components of all membranes. The role of plasma phospholipid levels on alteration of membrane properties is not well established. Farias et al. (1975) observed that diet altered the fatty acid composition of membrane lipids. Since the membranes of rat erythrocytes do not synthesize phospholipids, Guarnieri and Johnson (1970) considered their composition to depend upon the nature of the lipids of the diet. Membranes are capable of extensive acyl exchange of the fatty acids of their phospholipids (Van Deenen, 1981). Acylation-deacylation cycles tend to increase fluidity by substituting plasma polyunsaturated for saturated fatty acids in the 2-position; base exchange occurs
with both of the bilayers to alter the ratio of individual phospholipids; and, methylation of phosphatidylethanolamine alters its ratio to phosphatidylcholine with effects on membrane properties (Hirata and Axelrod, 1980).

Work with labeled compounds has shown a capability for exchange of membrane components. The cellular membranes of rat liver were shown to exchange complete molecules after administration of labeled phosphatidylcholine (LeKim et al., 1972) or phosphatidylinositol (Bloj and Zilversmit, 1977). The uptake of phospholipids in the myelin membranes of rats and bovines was catalyzed by cytoplasmic proteins (Brophy and Aitken, 1979). However, dietary alterations in phospholipids have not been shown to produce an alteration of membrane properties.

D. SIDE-EFFECTS

1. Choline

Since most of the research with large amounts of choline or lecithin is recent and preliminary, the dose-response characteristics are still ill-defined. Biological effects have been demonstrated only with large doses. The participants in the LSRO discussion agreed that 16-20 g/d of choline chloride approximated the highest tolerable dose. Some participants at the ad hoc review were aware of preliminary studies in which oral doses of choline chloride approximating 30 g may have produced cardiac arrhythmias.

The peripheral cholinergic effects of large doses of choline are nausea, vomiting, salivation, sweating, and anorexia. It may be observed that these effects are minimal compared to those produced by classical cholinomimetics such as physostigmine, oxotremorine, or arecoline. Choline effects are dose-dependent and can be counteracted by administration of muscarinic blockers such as propantheline, methscopolamine, etc. (Jenden, 1979). As noted on page 7, trimethylamine formed by action of bacteria in the intestine produces an objectionable body odor.

A number of cholinergic agonists have been reported to cause depression in susceptible individuals (Harris et al., 1979; Tamminga et al., 1976). Considerable animal data are available (Karczmar, 1979; Silbergeld and Goldberg, 1976) to indicate that cholinergic agonists may cause arrest of ongoing motor activity. Monkeys treated with cholinergic agonists have modified social interactions including development of aggression (Thomas et al., 1978). Such effects have occasionally been noted in patients treated with choline.
It is apparent that the amount of choline tolerated by a particular patient varies with the amount of bacterial destruction in the gut as well as with individual physiological characteristics. The participants in this LSRO review agreed that administration of maximal nontoxic doses of choline for therapeutic effects requires clinical supervision of the patients. When overt symptoms or beginning of depression are seen, the clinician can withdraw choline or adjust the dosage.

The initial finding of the effects of increased choline intake on postsynaptic cells was an increased activity of tyrosine hydroxylase in dopaminergic neurons of rat brain; the activity increased catecholamine synthesis (Ulus and Wurtman, 1976). Increases in tyrosine hydroxylase activity (Ulus et al., 1977a) and acetylcholine levels in the adrenal gland and several peripheral sympathetic ganglia (Ulus et al., 1977b) were observed. Choline failed to increase tyrosine hydroxylase activity when the corresponding preganglionic nerves were transected (Ulus et al., 1977a). This suggested that adrenal function is not affected by choline levels unless there is acetylcholine release. Thus, the postsynaptic effect of choline was not considered to be direct but involved a neuronally transmitted mechanism involving conversion of choline to acetylcholine and then its release. Oral administration of choline to rats (2.8 mg/kg) increased the urinary excretion of epinephrine, probably by enhancing the release of catecholamine from the adrenals (Scally et al., 1978). The investigators explained the results by assuming: a) an increase in the flow of impulses in presynaptic cholinergic neurons as a consequence of the increased level of choline; or b) an increase in the amount of acetylcholine released per nerve impulse after administration of choline. Both mechanisms may be active.

2. **Lecithin**

Lecithin has been administered as a source of both choline and linoleic acid and hence its physiological effects are similar to those of both compounds. The rationale for its use as a source of choline is its longer-lasting action and milder side-effects.

As much as 100 g/d of commercial lecithin have been administered to patients to provide therapeutic amounts of phosphatidylcholine (which constitutes only 20% of the product). The participants in the LSRO review reported that such quantities are not well accepted by many patients who object to the gritty character of the granular form, or to the large number of capsules of the fluid form that are required. In general, patients tolerate about 25 g of commercial lecithin without side-effects. Individual reactions to gradually increased doses include reduction in appetite, vomiting, abdominal bloating, belching, and diarrhea with fatty
stools (Etienne et al., 1978b). However, one ad hoc review consultant reported that 60-80 g phosphatidylcholine (85%) has been given to some patients without difficulty and almost all take 40 g/d without side-effects.

A diet in which lecithin was incorporated in the food was apparently well tolerated (Hirsch et al., 1978). Six human subjects ate three high lecithin meals equivalent to 5 g/d of choline for 2 consecutive days. The lecithin was provided by five egg yolks and 12 g of soybean lecithin per meal. Elevations in serum triglyceride and reduction in serum cholesterol levels were consistent with the high intake of unsaturated fatty acids provided by the soybean lecithin supplement. No significant increases in serum cortisol or prolactin were observed, suggesting that this mode of choline administration did not stress the subjects.

Another side-effect of high lecithin intake comes from the fatty acid component which may have the caloric equivalent of an extra meal. Intakes of 20-30 g/d for periods up to 11 mo by seven patients with hypercholesterolemia have been reported (Simons et al., 1977). The regimen was well-tolerated by all subjects, with temporary weight gains averaging 1 kg although one individual gained 6 kg.

Deposition of cholesterol in tissues and formation of gallstones have been suggested as potential hazards of long-term lecithin therapy (Grundy, 1975). The effect relates to the high linoleic acid content of soybean lecithin. High intakes of polyunsaturated fats were reported to cause an increased incidence of gallstones in men (Sturdevant et al., 1973). Vergroesen (1975) noted, however, that the stones had not been identified as containing cholesterol. Dam (1971) found soybean oil and cod liver oil produced amorphous pigmented stones in hamsters, and Melchior et al. (1972) reported gallstones in squirrel monkeys fed unsaturated fats. On the other hand, Toouli et al. (1975) gave 2.25 g of phosphatidylcholine plus 750 mg of cholic acid to nine patients with gallstones for 6 mo and produced a marked decrease in the lithogenic index*. The stone size decreased in three patients. Cholic acid alone was ineffective.

No changes in bile cholesterol or lecithin levels were found by Thistle and Schoenfield (1968) after ingestion of 20 g/d of lecithin by normal subjects but Tompkins et al. (1973) observed increased lecithin/cholesterol ratio in bile of patients with cholelithiasis on 10 g/d lecithin. Cobb et al. (1980) found similar results with healthy volunteers fed 22.5 g/d lecithin for 4 wk.

* The lithogenic index is defined as the ratio of cholesterol actually present to the maximum amount that would be soluble at the actual bile salt-phospholipid ratio of the bile (Thomas and Hofmann, 1974).
Significant amounts of choline are not released from phosphatidylcholine in the lumen of the intestine so that bacterial production of trimethylamine is not a problem. It is not known what percentage of massive doses of ingested lecithin escapes absorption. Clinical supervision of high lecithin intakes is necessary, according to the ad hoc review group, to adjust the diet to compensate for the caloric content of lecithin as well as to monitor the patients for cholinergic side-effects, particularly depression.

High intakes and the attendant side-effects could be reduced if preparations with a high content of phosphatidylcholine were readily available. Presently such preparations containing 85–95% phosphatidylcholine have been provided by some manufacturers, but only on a limited basis for research (see footnote, Wurtman, 1980, p.74).

The cardiac pathological lesions obtained in rats after a diet of rapeseed oil have been attributed to the erucic acid (C_{22:1}) content (Beare-Rogers, 1975). This has raised questions about the effects of phosphatidylcholine or lecithin from rapeseed and containing fatty acid moieties which might include significant amounts of erucic acid (Hanin, 1979). The participants in the group discussion agreed that lecithin containing eicosa- or docosamonomonoenoic acid was tolerable if the content of such fatty acids was less than 6%.

E. POTENTIAL EFFECTS ON THE NERVOUS SYSTEM

1. Adaptation

The distribution of cholinergic, autonomic, and somatic innervation is well-known. Peripheral cholinergic innervation extends to skeletal and smooth muscle including the vascular system and the intestine. Also connected are the exocrine and endocrine glands, lacrimal and nasopharyngeal glands. Cholinergic synapses impinge on other neurotransmitters in the central nervous system such as dopaminergic, serotonergic, GABA-ergic systems. Thus, perturbation of cholinergic function will be reflected by adaptation in the other neurotransmitter systems, but the total extent of the interaction is not known. Of further concern are the effects on cyclic nucleotides that may serve as second messengers for neurotransmitter action. Present data are insufficient to determine the effects of a cholinergic diet on such susceptible physiological and behavioral actions as aggression, depression, temperature control, food and water intake, drug tolerance, sensory processes, diurnal rhythms, sleep patterns, analgesia, and sexuality (Karczmar, 1979). That such effects have not been reported generally after ingestion of large amounts of choline or lecithin may possibly be attributed to close supervision of the patients under clinical trials.
Karczmar (1979) has suggested that a cholinergic diet possibly could lead to postsynaptic changes which might lead to progressive refractivity of the myasthenic endplates to anticholinesterase therapy. If acetylcholine accumulation, such as can be induced by anticholinesterases, could be produced by a high lecithin diet, desynchronization might be supplanted by generalized EEG seizures and motor convulsions.

Long-term cholinergic stimulation may affect peripheral sites such as transport processes in the intestine (Browning et al., 1977), the phospatidic acid-dependent sodium pump and phosphorylation processes (Hokin and Hokin, 1960), possible neurotrophic processes (Drachman, 1976), placental changes (Murthy et al., 1977), and sperm motility (Bishop et al., 1977). Vergroesen (1979) suggested that ingestion of large amounts of lecithin, by virtue of its linoleic acid content, may stimulate prostaglandin (PGE) synthesis. PGE₁ and PGE₂ can antagonize the inhibitory effects of norepinephrine by blocking the effect of adenylcyclase in the heart, adipose tissue, and cerebellum. Also PGE may affect thermoregulatory centers of the hypothalamus.

2. Withdrawal

A relationship between increased cholinergic activity and dopamine transmission in the substantia nigra and corpus striatum mediated by a GABA-ergic (γ-aminobutyric acid) neuron has been proposed (Groves et al., 1975). According to this model increased cholinergic activity would result in decreased dopaminergic transmission. This hypothesis raised the possibility that chronic treatment with a cholinomimetic agent in such conditions as tardive dyskinesia or memory loss could ultimately produce a hypersensitive dopamine receptor (Davis and Rosenberg, 1981). Withdrawal of the agonist could precipitate a tardive dyskinesia-like syndrome. Alternatively, an adaptation to high lecithin intake could obviate this result by adjustments in brain choline and/or acetylcholine presynaptic levels. While withdrawal symptoms are a concern, there is insufficient clinical or experimental evidence to assess their significance. The possibility remains highly speculative and is based on preliminary animal studies with diisopropylfluorophosphate, a very potent acetylcholinesterase inhibitor.
IV. BEHAVIORAL AND NEUROLOGICAL EFFECTS OF ORAL ADMINISTRATION

A. THEORETICAL ASPECTS

1. Precursor approach

The treatment of neurological disorders associated with inadequate cholinergic function was developed as an extension of the "neurotransmitter replacement approach" which originated in the use of levodopa for treatment of Parkinson's disease and was extended to other neurotransmitters (Growdon and Wurtman, 1979). It was proposed that choline administered parenterally, or by diet, would increase acetylcholine synthesis. The therapeutic value of increased acetylcholine levels was established by use of cholinesterase inhibitors (Tanner et al., 1979). Many of the clinical investigations have been empirical. Nevertheless, the principle of precursor loading to potentiate neurotransmitter synthesis has gained much support in a rapidly expanding field of investigation (Jenden, 1979) but many theoretical and practical questions remain.

The rationale of the treatment was established by Cohen and Wurtman (1975) who showed that injected choline increased the brain acetylcholine in rats. The use of choline as a precursor was first tested on a patient afflicted with tardive dyskinesia (Davis et al., 1975). Subsequent experiences in clinical testing are reviewed in this section.

Choline is not an ideal precursor of acetylcholine because the compound is quickly cleared from the plasma and tissues. Thus, the period of effectiveness is limited. Demonstration that choline was effective when administered orally (Cohen and Wurtman, 1976) was soon followed by the finding that oral lecithin administration provided a longer acting, better tolerated precursor of choline (Wurtman et al., 1977).

Growdon and Wurtman (1979) have proposed a set of criteria for predicting conditions under which a dietary precursor may affect neurotransmitter synthesis:

(a) the brain cannot synthesize as much of the precursor as it may need;

(b) the level of the precursor in the plasma must fluctuate with intake, i.e., not be subject to feedback inhibition;

(c) the rate of transport of the precursor into the brain must be a function of precursor concentration in plasma, i.e., a low-affinity system that is not saturated under normal conditions; and,
(d) the enzyme that catalyzes the rate-limiting step in neurotransmitter synthesis must also be unsaturated and therefore responsive to changes in precursor availability, i.e., the enzyme activity must not be limited more by some scarce cofactor than by the precursor, or be subject to feedback inhibition.

The criteria with regard to acetylcholine synthesis have been extensively investigated. The first two criteria are well-established but there is still discussion regarding the latter two (Jenden, 1979). Wecker and Dettbarn (1979) recently reported no change in hippocampal or cerebral cortical acetylcholine levels of rats after choline chloride administration (60 mg/kg i.p.) although there was an increase in the striatum in the presence of an anticholinesterase, paroxon.

The interrelationships between the high- and low-affinity systems which transport choline into the brain are still undefined. The high-affinity system is saturated at usual brain choline concentrations and accounts for most of the acetylcholine synthesized. One possibility is that elevations of extracellular choline promote synthesis of acetylcholine only at sites where the low-affinity transport system is operating (Jenden, 1979). The fundamental question whether brain acetylcholine levels are modulated by plasma levels of choline is supported by the experiments of Ulus et al. (1977a). They showed that choline chloride administration to rats produced transsynaptic induction of adrenal medullary tyrosine hydroxylase. Haubrich et al. (1979) found that repeated administration of low doses of choline was effective in inducing increases in dopamine metabolism to form homovanillic acid.

2. **Direct agonist action of choline**

The pharmacologic potency of choline varies from 1/100 to 1/100,000 that of acetylcholine, depending upon the system observed but it is 1:10 to 1:20 for muscarinic receptors in the brain (MacIntosh, 1979). Eckernäs et al. (1977) have considered the possibility that choline might function directly as a cholinergic agonist. From turnover studies on acetylcholine in rat striatum and cortex they concluded choline might be functioning as a cholinergic agonist in striatal neurons. Ladinsky et al. (1979) showed that i.p. administration of a large dose of choline iodide, which elevated the plasma level approximately twelvefold, raised the acetylcholine of the brain only 20-25%. Prior administration of atropine counteracted the increase in striatal acetylcholine. The blockage by atropine (or reserpine) but not by atropine methihexital, which does not cross the blood-brain barrier, was interpreted as indicating that a rise in acetylcholine content may result from the action of choline as an agonist at central muscarinic receptor sites.
Krnjevic and Reinhardt (1979) applied choline iontophoretically to the pericruciate cortex of cats and concluded that large systemic doses of choline can excite neurons by direct action through muscarinic receptors. Iontophoretic applications of choline were eight times weaker than applications of acetylcholine in causing firing of neurons in the cerebral cortex of cats. The excitatory effect of choline, like that of acetylcholine, was suppressed by atropine but not by hemicholinium-3 or triethylcholine, agents which block choline uptake. The action was not potentiated by phystostigmine which greatly potentiates the action of acetylcholine.

Sitaram et al. (1979) compared the effects of choline on memory and sleep of normal volunteers and suggested that choline may exert postsynaptic effects on both nicotinic and muscarinic sites.

The hypothesis that choline acts indirectly to release preformed acetylcholine in the neuron was refuted by Bierkamper and Goldberg (1979). They perfused rat hemidiaphragm phrenic nerve preparations with diisopropylfluorophosphate to inhibit cholinesterase and then subjected them to 7 Hz stimulation to release acetylcholine at a steady rate (6 pmoles/min). The amount of acetylcholine released varied with the concentration of choline in the medium. Comparison with controls (no choline) showed that exogenous choline was being utilized. Choline supplementation did not increase nonstimulated release of acetylcholine to levels equivalent to the stimulated output. The investigators concluded that choline acted by participating in the synthesis of acetylcholine and not by either direct agonist action or release of preformed neurotransmitter.

Conversely, Löffelholz et al. (1979) observed that the chicken heart, isolated rat superior cervical ganglion, or rat intestinal smooth muscle released acetylcholine continuously during a 15-20 min nerve stimulation. During this period the acetylcholine content of the tissues was only slightly decreased even without addition of exogenous choline to the perfusing medium. When 10^{-5} M choline was infused into a chicken heart, the evoked outflow of acetylcholine doubled. This finding supports the hypothesis that extracellular choline is a determinant for synthesis and release of acetylcholine from central and peripheral neurons and its availability becomes more important when neurons increase their firing rate.

B. AGING AND MEMORY

Aging begins at birth. The first phases, growth and development, continue well beyond puberty. After this period, aging changes are generally considered to be degenerative.
Memory is the process of reproducing or recalling what has been learned or experienced. Learning is thought to involve an association among structural elements of the nervous system so that an adaptive response can be made to a new environmental stimulus (short-term memory). This is followed by a consolidation process that leads to more or less permanent fixation of the new neuronal association (long-term memory). A facilitation of transmission across synaptic endings insures that the environmental stimulus will elicit the adaptive response in the future. The seat of memory in the brain has never been identified. Storage of information in the central nervous system is probably distributed over large regions of the neural network (Glassman, 1969).

1. Effects on memory and cognition in aging

Within the last decade, considerable information has accumulated implicating central cholinergic neurotransmitter mechanisms, which may be particularly impaired with age, to be partially responsible for geriatric memory deficits. The cholinergic hypothesis of impaired memory is supported by corroborative pharmacological, electrophysiological, and neurochemical evidence. The pharmacologic evidence derives from observations that blocking central mechanisms with scopolamine impairs memory in young subjects in a manner uniquely resembling memory loss in aged subjects (Drachman and Leavitt, 1974). Neurochemical findings have demonstrated that the brains of aging subjects exhibit decreases in density of muscarinic receptors and of choline acetyltransferase activity (White et al., 1977). Electrophysiological experiments show functional impairment of cholinoreceptive neurons in aged rats with memory deficits (Lippa et al., 1980).

Participants at the LSRO ad hoc review group meeting agreed that memory changes need not be always related to the aging process. The dual mechanisms of memory storage and recall are not well known. Although other factors such as hormones, neuropeptides, and RNA synthesis may affect memory, the cholinergic aspects have stimulated much research toward improving memory by administering choline, its precursors, or drugs that stimulate intraneuronal production of acetylcholine.

Assessment of the results of various investigations on the effects of choline and lecithin on cognitive function and memory requires correlation of the psychological, behavioral, and environmental methods. Hingtgen and Aprison (1976) have reviewed behavioral and environmental testing methods used with animals. Psychological tests for memory and cognition in man are numerous (Postman, 1976). There are well-recognized difficulties in extrapolating animal memory training tests to human psychological assessments.
a. Animal studies. Studies with aged, memory-impaired animals show that significant changes which occur in the cholinergic mechanisms with aging are partially responsible for loss of memory (Deutsch, 1979).

Deutsch (1971) noted that acetylcholine is rapidly destroyed by an anticholinesterase during transmission at synapses in rats. Anticholinesterase drugs such as physostigmine, by inactivating the cholinesterase, prevent destruction of acetylcholine. Deutsch proposed that the overall effect of submaximal levels of anticholinesterase could be an increase in concentration of acetylcholine at the synapse with improvement in the efficiency of the transmission; excessive levels could produce a synaptic block. Thus, if changes occur in acetylcholine emitted at synapses modified by learning, such synapses should show either facilitation or block depending on the amount of anticholinesterase administered.

Brain injections of anticholinesterases such as physostigmine or diisopropylfluorophosphate had little effect on memory of the rat for running a maze if the drug was injected 3 d after training. If injected 5 or 14 d after training the rat had partial or complete amnesia, respectively. By 28 d the learned behavior had been forgotten, but recall was enhanced by an anticholinesterase which increased acetylcholine levels at the synapse. The anticholinergic agent, scopolamine, had an opposite effect. Deutsch (1971) also recognized that memory strength is another variable affecting maze performance. Weak, poorly-learned habits were greatly facilitated and strong, well-learned habits were blocked by injections of an anticholinesterase. He suggested that two phases are present in memory storage: modification of cholinergic synapses during learning, with development of increasing sensitivity of the postsynaptic membranes to acetylcholine and, after a certain period, decline in sensitivity instituting the phenomenon of forgetting. Passive avoidance retention tests with rodents showed the "strength" of a memory depends upon intensity of training, difficulty of concept, or period of decline (Deutsch, 1979). Weak memories produce poor conduction in cholinergic synapses while strong memories cause more efficient conduction. The anticholinesterase effect is due to synaptic blockade by excess acetylcholine. Thus, as stated earlier (Deutsch, 1966), "retrograde amnesia is (hypothetically) due to cholinergic insufficiency".

Bartus et al. (1981) observed that pharmacologic manipulation of other neurotransmitter systems also alters retention of newly acquired behaviors. Many investigations, however, provide evidence that the cholinergic system may have a significant role in age-related memory deficits. Of particular note is the memory deficit produced by blockade of central muscarinic receptors in young subjects, which were similar to those found naturally in the aged.
Passive avoidance retention tests have given additional information on rodent memory. Bartus et al. (1980a) showed that aged mice, 23 mo or older, suffered impairment in learning and memory. Old mice on a high choline diet performed as well as 3-mo-old animals. Two other behavioral indices, motor and psychomotor activity, that exhibited significant changes with age, were not affected by choline manipulations. These results suggested that age-related changes in memory and learning might be ameliorated by increased choline intake. Loss of cholinergic receptors and decrease in choline acetyltransferase activity are natural consequences of aging in rats (Strong et al., 1980) and in mice (Lippa et al., 1980).

However, Bartus et al. (1980a) compared choline with two other cholinomimetics (physostigmine and arecoline) on performance of a memory task in aged monkeys. Although both physostigmine and arecoline induced statistically reliable effects, choline was not found to be effective, even though 8000 times more choline was administered than the acknowledged cholinergic agonist, arecoline.

The effects of physostigmine on recent memory in young (5-7 yr) and aged (18+ yr) rhesus (Bartus, 1979) and cebus (Bartus et al., 1980a) monkeys were similar to those on young humans described below (Davis et al., 1978); e.g. there were no effects at low doses of physostigmine (1.3 μg/kg), some improvement at a restricted intermediate range of doses, and deficits at the highest dose (40 μg/kg). Arecoline, a muscarinic cholinergic agonist, produced more consistent effects on memory performance at 50 and 75 μg/kg intramuscularly in cebus monkeys. However, choline administration at 30-400 mg/kg in the same animals failed to produce substantial increases in performance. Neither dopamine agonist, apomorphine (25-200 mg/kg), nor levodopa (25-220 mg/kg), was effective.

On the basis of reports that oxidative metabolism is decreased in brains of humans after the sixth decade, Bartus et al. (1980b) administered choline or piracetam, a drug purported to enhance energy metabolism, to aged rats known to suffer deficient memory and tested them for retention of passive avoidance learning. Choline caused no significant improvement in performance; piracetam resulted in better retention times, but a combination of choline and piracetam gave several times better results.

Aged, memory-impaired rhesus monkeys were tested with three drugs which are considered to enhance energy metabolism (Bartus and Dean, 1981). The dosing was done over 9 d with each animal receiving, in turn, piracetam, vincamine, and dihydroergotoxine in two dose levels. Some improvement in performance was noted, but the results were variable and, in some cases, mild impairment was noted. Similar results were seen in aged cebus monkeys dosed with piracetam and centrophenoxime. The investigators felt the results encouraged further investigation.
b. Human studies. Impairment of learning and memory, particularly short-term memory, in man is a well-known occurrence accompanying the aging process. Choline and lecithin have been fed to demented patients in efforts to improve cognition and memory. In addition some effort has been made to improve memory in younger, healthy individuals. Accounts of such research in the news media have stimulated public interest in choline and lecithin as dietary supplements. Capacity for memory is prized by individuals of all ages; gradual loss of memory is lamented by the elderly.

There is some disagreement about the relationship between the memory loss observed in persons with mental disorders and the loss in normal elderly persons. One point of view is that senile dementia represents an extreme of the loss of the cholinergic neuronal activity that occurs in normal aging (see page 43). Others believe that some features of degeneration in Alzheimer's disease are unique to the disease (Bowen et al., 1979; Smith and Swash, 1978). Nevertheless the rationale of treatment with cholinergic precursors to restore memory is based on the same principle for normal aged as for those with mental disorders.

Participants in the LSRO review agreed that aged individuals do not respond to acetylcholine stimulation as well as the young. Supposedly the elderly have a poorer neuron firing rate as well as reduced receptor density and binding affinity.

In a number of trials, choline treatment failed to improve memory in the aged. Ferris et al. (1979) reported an effort to improve the memory of 14 elderly outpatients, who were suffering from mild to moderate cognitive impairment, by oral administration of choline chloride in gradually increasing doses to a maximum of 12-20 g/d. The highest dosage was maintained for 2 wk. None of 26 cognitive tests measuring both memory and performance showed statistically significant improvement in any of the patients. Mohs et al. (1979) gave 16 g/d of choline chloride orally for 4-8 d to eight normal, elderly subjects (64-84 yr) in a double-blind study. There was no significant difference from controls in performance of tasks involving storage and retrieval of information. On the basis that the dosage of choline might have been too high or of insufficient duration, Mohs et al. (1980) screened healthy subjects to select 10 responders to a memory test. The subjects (mean age 68.5 yr) consumed 2 g of choline four times a day for 20 d with appropriate placebo controls. Tests designed to measure both storage and retrieval of information were administered at intervals during the trial. Choline administration did not improve the performance of any of the subjects.

The investigators observed that the failure of choline to improve the memory of nondemented elderly patients is contrary to expectations. As noted below, drugs such as physostigmine that enhance central cholinergic activity have improved memory in both
young (Davis et al., 1978) and elderly (Davis et al., 1979a) although there have been failures as described above. However, the successful use of choline in combating neurological motor disturbances may have been its effect on the striatum, whereas drugs that affect memory are believed to act on the hippocampus. Also in normal patients cholinergic function may be near optimal levels.

2. Effects on control subjects

Many of the studies dealing with the cholinergic system in human memory have utilized scopolamine or atropine which block muscarinic acetylcholine receptors (Drachman and Sahakian, 1979). Drachman and Leavitt (1974) and Drachman et al. (1980) found that scopolamine impaired storage of new information, non-memory cognitive function, and possibly retrieval in young, normal subjects, but had no effect on immediate memory span. There were marked similarities to the decreased memory storage and cognitive function in undrugged, elderly individuals (59-89 yr).

Physostigmine (1 mg s.c.) did not produce significant changes in memory or other cognitive functions in the young subjects (19-25 yr) when compared with normal controls; although there was a trend toward improvement, 2 mg physostigmine produced a trend toward impairment of function.

Infusion of 1.0 mg physostigmine i.v. into 19 normal males (18-35 yr) produced enhanced storage of information in long-term memory and improvement of recall of previously learned words (Davis et al., 1978). A test with 3 mg worsened recall (Davis et al., 1976a). The capacity for short-term memory was significantly diminished. Peters and Levin (1977) reported a case history of a young woman suffering from memory deficit, secondary to postherpes encephalitis, who had a marked enhancement in consolidation and recall of long-term memory after 0.8 mg physostigmine s.c., but no benefit from higher or lower doses. Thus, physostigmine may improve or impair some memory functions depending on the dose.

Intramuscular injections of arecoline were given to 14 subjects (mean age 22.4 yr) (Sitaram et al., 1978). All were treated with methscopolamine (0.3-0.5 mg) to counteract peripheral cholinergic side-effects. Subjects receiving scopolamine alone had impaired free recall of previously learned words, whereas arecoline (4-6 mg) enhanced recall and also counteracted scopolamine effects. The investigators administered 10 mg of choline orally to 10 subjects (mean age 24 yr) previously trained to recall a list of 10 words. Two of the subjects had improved performance after the choline. The poor performers in the test showed relatively greater improvement than good performers after they received arecoline or choline and greater impairment after scopolamine.
Altogether the results of the studies reported above indicate that there is empirical support for precursor manipulation of the cholinergic system to improve memory. However, at present it is questionable if choline can improve memory in normal subjects. Davis et al. (1980b) fed 15 males, who had previously received improvement in memory and recall from physostigmine, 16 g/d of choline chloride for 3 d. An inverse correlation was found between a subject's response to physostigmine and choline when short- and long-term memory functions were evaluated. Nine males (20-30 yr) from the previous study were given 8 g/d of choline chloride orally for 3 d (Mohs and Davis, 1980). Choline had no significant effect on average performance either on a test of memory storage or of memory retrieval. Correlational analysis indicated, however, that subjects who improved most from 1 mg of physostigmine tended to show slight improvement from choline administration.

C. OBSERVATIONS FROM CLINICAL USE IN TREATMENT OF DISORDERS

Choline and lecithin have been used in preliminary studies for the treatment of a variety of movement disorders. Some improvement in some patients has been reported in the signs and symptoms of tardive dyskinesia, Gilles de la Tourette's disease, Friedreich's ataxia, and levodopa-induced dyskinesia. Improvements in the conditions of patients with Huntington's or Alzheimer's disease have been limited to a few patients. Other conditions such as spastic spinocerebellar degeneration, mania, and the myasthenic syndrome have been studied only in a limited number of patients.

1. Tardive dyskinesia

This disorder is a hyperkinetic, neurologic disorder characterized by involuntary choreiform movements of the lips, tongue, jaw, and occasionally, the extremities (Fann, 1980; Freedman, 1973). The condition can occur in susceptible persons after as little as 3 mo of treatment with neuroleptic drugs such as the phenothiazines or butyrophenones but is usually observed after several years (Freedman, 1973). The mean reported prevalence is 15-20% in institutionalized patients such as schizophrenics. The exact incidence of tardive dyskinesia is not known and estimates of its prevalence range from 5-40% (Fann et al., 1972).

Tardive dyskinesia may be the manifestation of a drug-induced hypersensitivity of dopamine receptors in which dopaminergic response overrides the normal balance between dopaminergic and cholinergic function in the basal ganglia (Baldessarini and Tarsy, 1980). Theories of the mechanism are still controversial. Brains of patients with tardive dyskinesia do not show gross structural changes, but the condition is more prevalent in the elderly in whom neuronal loss commonly occurs.
Many pharmacological agents that have been tested to correct the imbalance have shown unsatisfactory results. These include compounds which block the action of dopamine, prevent its synthesis, deplete its stores, or inhibit dopaminergic neurotransmission (GABA agonists). The results of extensive clinical investigations have been the subject of several comprehensive reviews (Barbeau, 1978a; Berger and Rexroth, 1980; Davis et al., 1979b, 1980a; Fann et al., 1980; Growdon and Wurtman, 1981).

Choline and lecithin have been employed as alternative experimental treatments for the disorder. Limited clinical trials indicate the administration of choline or lecithin is accompanied by improvement in the course of tardive dyskinesia in some but not all patients. The significance of the improvement is often uncertain because the methodology of assessment is still under development. Study of tardive dyskinesia is confounded by observations that some patients may have a gradual spontaneous recovery regardless of treatment modality.

Davis et al. (1975) reported reduction in choreiform movements in a patient given 16 g/d of choline chloride. The effects persisted for 3 d after the treatment was stopped. Subsequently, similar results were obtained with three of four additional patients studied under double-blind conditions (Davis et al., 1976b). The three responders also had a transient remission after 3 mg physostigmine were given intravenously. Davis (1980a) postulated that response to physostigmine may predict response to choline. Tamminga et al. (1977) also confirmed these results in four additional patients. Some patients improved after intravenous physostigmine and choline. In a double-blind crossover study (Growdon et al., 1977a), 9 of 20 patients were significantly improved by ingestion of 8-20 g/d of choline. Plasma choline levels in the patients rose 170% during the treatment. In subsequent studies Barbeau (1978b) reported suppression of tardive dyskinesia in two patients and Yahr (1978) reported experience confirming the efficacy of choline.

Growdon et al. (1978) reported that 40-80 g/d of lecithin (20% phosphatidylcholine) or partly purified phosphatidylcholine increased plasma choline levels and decreased abnormal movements in three patients. Barbeau (1979) conducted a single-blind study in which 33 g/d of lecithin was given to four patients for 20 wk. Choreiform movements were reduced an average of 52%.

Jackson et al. (1979) observed significant improvement in six patients who received 50 g/d of lecithin (60% phosphatidylcholine). Branchey et al. (1979) obtained a decrease in mean frequency counts of abnormal movements in four of seven patients who were given a single dose of egg lecithin (60% phosphatidylcholine), but did not observe significant improvement by two rating scales when the total study population was analyzed. Gelenberg et al. (1979a) compared results of treatment of five patients for 6-8 wk.
with choline (150-200 mg/kg/d) and lecithin (eight patients, 21 g/d of 20% phosphatidylcholine slowly raised to 105 g/d) over a period of 6-8 wk. All patients improved on either agent and there was a tendency toward better results on lecithin. Two patients on lecithin developed mild and transient parkinsonian signs.

The bulk of currently available evidence strongly indicates that choline or lecithin administration does suppress choreic movements in many patients with tardive dyskinesia. However, Davis et al. (1979b, 1980a) have pointed out that choline chloride does not remove all the manifestations of the disease. Patients show a reduction in abnormal movements but retain a clinically noticeable dyskinesia. The effectiveness of choline and of lecithin in the treatment of tardive dyskinesia encourages further study.

Dimethylaminoethanol (deanol) has been used to treat tardive dyskinesia because it has been reported to increase brain acetylcholine content and perhaps cholinergic activity (Haubrich et al., 1975a). Casey (1977) and Domino and Kovacic (1980) after reviewing available data concluded that the effectiveness of deanol in treatment of tardive dyskinesia is currently unresolved. Of 161 patients treated with deanol in 28 investigations, 59 improved (37%), 1 worsened, and 101 (63%) showed only a mild response or no change. If blind- and placebo-controlled studies alone are considered, only 16 patients received benefit from their treatments. Subsequent studies with deanol have given equally equivocal results (Cole et al., 1980; Davis et al., 1980a).

A recent volume (Fann et al., 1980) reviews animal models of dyskinesia in monkeys, rats, mice, and guinea pigs, which have been produced by long-term administration of neuroleptic drugs. No model appears to mimic human tardive dyskinesia completely but all have been useful in providing insight into the causative mechanisms.

2. Huntington's disease

Huntington's disease, or chorea, is similar symptomatically to tardive dyskinesia but has a genetic basis with dominant inheritance. It is progressive and is characterized by personality changes, mental deterioration, slurred speech, involuntary muscular contractions (chorea), and unsteady gait. Death usually occurs 12-15 yr after appearance of the first signs or symptoms (Perry et al., 1973).

Pathologically, Huntington's disease is characterized by marked atrophy and neuronal loss in the basal ganglia with diffuse neuronal loss in the cerebral cortex, particularly in the frontal lobes. Fine structure examinations of cortical neurons in Huntington's disease have shown large accumulations of lipofuscin and disorganization of intraneuronal membranes (Goebel et al., 1978).
Biochemical studies have shown the concentrations of GABA (Perry et al., 1973) and glutamic acid decarboxylase are reduced in the basal ganglia (McGeer et al., 1973; Stahl and Swanson, 1974). A loss of choline acetyltransferase, γ-glutamic acid decarboxylase, and a marked decrease in muscarinic cholinergic and serotonergic binding sites occur in the caudate nucleus but not in the cerebral cortex (Enna et al., 1976; Wu et al., 1979). Spokes (1980) found increased dopamine and norepinephrine in several regions of the brains at autopsy of patients with Huntington's disease. GABA was decreased in all regions and there were losses of choline acetyltransferase in the striatum, nucleus accumbens, septal nuclei, and hippocampus. The neurons in the cerebral cortex showed abnormal histone distribution (Goebel et al., 1978; Iqbal et al., 1974).

Davis et al. (1976b) gave choline chloride orally to six patients with Huntington's disease. The dosage was started at 1 g, four times per day and increased every 2-3 d until an intake of 20 g/d was reached and maintained for 3-4 wk. The following 4 wk the patients were given placebos followed by a second trial of 20 g/d of choline chloride for 4-8 wk. Only two patients improved significantly in movement disorders during the choline treatment but two others had a slight improvement. The signs and symptoms returned during the placebo period.

Growdon et al. (1977b) treated 10 ambulatory patients with 8-20 g/d of choline for periods of 21-124 d. In eight of the patients choline was given in combination with other drugs, e.g. haloperidol, lithium, or flufenazine. None of the signs of Huntington's disease were suppressed although there were minor benefits in speech and gait in a few patients. Similarly, Aquilonius and Eckernäs (1977) found choline chloride, 3-15 g administered orally, did not conclusively alter the involuntary movements in five patients but those who showed some response to treatment were also benefited by infusion of physostigmine. Eckernäs and Aquilonius (1979) administered 3 g/d of choline chloride orally to five patients and increased the dose in 3 g increments every 4th day to 12-15 g/d for a total period of 1-2 mo. At the highest dose level (15 g) the frequency of hyperkinesia in five of nine patients was reduced by a mean 56% of the premedication level. Two patients were tested with an unspecified amount of sodium phosphorylcholine for 21 d. Some improvement in choreiform movements was observed. The investigators observed that phosphorylcholine did not produce a "fishy" body odor. Barbeau (1979) found that treatment of five patients with 19.5 g lecithin (20% phosphatidylcholine) for 19.6 wk gave only mild improvement in abnormal movements (25-29%).

Results from treatment of Huntington's disease with choline have not been encouraging to further studies. Lecithin administration has not been pursued as a substitute. The consultants observed that choline therapy could not be expected to
ameliorate Huntington's disease if there was a large deficiency of receptive cholinergic neurons in the brain. The patients who have shown some benefit from the treatment are those whose disease had not progressed very far and, in general, were temporarily improved by physostigmine.

An animal model for research on Huntington's disease has been proposed by Coyle and Schwarcz (1976) and by Mason and Fibiger (1979). The model is based on destruction of striatal neurons by infusion of kainic acid into the dorsal striatum of rats and mice. The anatomical and biochemical lesions resemble those of Huntington's disease (McGeer and McGeer, 1976). In the rat a twofold increase in nocturnal locomotor activity was observed as a definitive sign.

3. Alzheimer's disease

Dementia characterized by a generalized cerebral atrophy with histological features of a large increase in senile plaques, neurofibrillary tangles, and granulovascular degeneration is termed Alzheimer's disease (Yates et al., 1980). In strictest terms Alzheimer's disease refers to the presenile form of the disease with onset before 65 yr while onset after 65 yr, the senile form, is called Alzheimer-type disease (Terry, 1978; White et al., 1977). However, many investigators believe Alzheimer's disease is a variant of simple senile dementia resulting from common degeneration of the central nervous system with aging. Katzman et al. (1978) have published a comprehensive review of the disorder. Smith and Swash (1978) consider that granulovascular neuronal degeneration distinguishes Alzheimer's dementia from the normal aging process. The rate of loss of neurons in Alzheimer's disease generally exceeds the slow decrease in healthy elderly subjects.

Clinical abnormalities center about intellectual deterioration and failure of memory. A prominent early characteristic is a loss of recent memory. Attention span is limited and concentration is faulty. Even simple acts become difficult to perform. Thinking in the abstract is lost; judgment becomes defective. Epileptic seizures and myoclonic twitchings may occur.

The role of the cholinergic system in Alzheimer's disease has been reviewed recently (Perry and Perry, 1980). The principal biochemical lesion is a dramatic decrease in choline acetyltransferase activity in the hippocampus (Bowen et al., 1976, 1979). The decrease is much greater than in the normal elderly. Growdon and Corkin (1980) have summarized seven investigations which confirm this finding and establish that binding by cholinergic receptors is normal. These considerations led to tests of choline and lecithin in treating patients with presenile and senile dementia.
Because the condition involves progressive degeneration, investigators have tended to choose patients in early stages of dementia for their trials. Some of these patients have had limited improvement in the signs and symptoms of Alzheimer's disease.

In the first study, Boyd et al. (1977) gave 5 g/d of choline chloride orally for 2 wk and then 10 g/d for 2 wk to seven patients (aged 70-80+ yr) with advanced senile dementia. Nurses noticed some improvement in the patients' social behavior but there was no improvement in cognitive performance.

Etienne et al. (1978b) gave choline bitartrate orally for 4 wk to three patients with moderately advanced Alzheimer-type senile dementia. The dose was increased to 8 g/d (as choline) in 1 g increments every 2 d. Only the patient who was least demented showed an improvement which was in constructional ability. The improvement coincided with her peak plasma choline level. One patient became incontinent for the duration of the test. Smith et al. (1978) gave 9 g/d of choline bitartrate for 2 wk to ten Alzheimer-type patients, mean age 77 yr. No improvements were noted in a battery of tests of behavior and cognition. Three patients showed evidence of heightened awareness of their surroundings. There was an exacerbation of urinary incontinence in three patients.

Two Alzheimer-type patients (ages 73 and 78 yr), and six with Alzheimer's disease (ages 58-66 yr), were given 9 g/d of choline citrate by mouth for 21 d (Signoret et al., 1978). Two series of psychological tests were administered. Efficiency of relearning and recall of 30 pictures improved slightly in the younger patients with short disease duration, but the performances were poorer than those of eight normal controls. Manual motor learning was not improved. Povall et al. (1980) conducted a double-blind, placebo-controlled study with choline bitartrate in five patients who had mild to moderate Alzheimer's disease. Oral doses of 8, 12, and 16 g/d of choline bitartrate were given for 2-wk periods interspersed with two 2-wk placebo periods. A cognitive test battery of 10 tasks was conducted at the end of each week. At 12 g/d, choline bitartrate produced enhanced performance on word recognition tasks. At this intake the mean plasma choline level was nearly double the controls.

Etienne et al. (1978a) gave 25 g/d of lecithin (23% phosphatidylcholine) to seven patients (42-81 yr, mean age 67 yr) for 4 wk. Three patients responded to treatment. By clinical impression they seemed to understand instructions better, were more cooperative, and displayed less striking speech rambling. However, the improvement ceased after lecithin was stopped. Psychological tests showed no choline-dependent changes in memory, constructional ability, or face recognition.
Christie et al. (1979) compared the effects of choline and lecithin in 11 Alzheimer's disease patients. Treatment began with 1 g/d of choline chloride and increased over a 4-d period to 5 g/d for 5 d. Choline was then withdrawn and the patients were kept on a choline-free diet for 7 d. Thirty grams of lecithin granules (20-30% phosphatidylcholine) were then given, increasing to 100 g/d in 4 d. The treatment was continued for 3 mo on an outpatient basis. No patient had urinary incontinence but the high dose of lecithin caused diarrhea and two patients had fecal incontinence. Some patients were mildly irritable and treatment of one had to be discontinued because of aggressive behavior. No improvement in patient test performances was observed during choline administration. Three of the nine patients who completed the trial on lecithin showed considerable improvement in speech and topographical orientation. Neither of the two more severely affected patients improved. Base mean plasma choline levels were 14.2 ± 4.3 nmole/ml which rose to 32.6 nmole/ml on either choline or lecithin intake. Psychological testing showed two patients improved after their 6-wk ward treatment but neither improvement nor deterioration could be detected at the end of the 3-mo outpatient period. The two patients who dropped out of the test deteriorated further.

Peters and Levin (1979) gave five Alzheimer's disease patients physostigmine s.c., a placebo, and then 1.2 g of lecithin three times per day to three of the five. Memory tests showed improvement in all three patients receiving both physostigmine and lecithin. Neither physostigmine nor lecithin alone gave comparable results. Smith and Swash (1979) administered 1 mg of physostigmine salicylate s.c. to an Alzheimer's disease patient who had only 23% of the choline acetyltransferase activity of control brains. The drug produced no improvement in the number of correct responses in a battery of memory tests but did reduce the number of inappropriate responses in three tests.

The possible efficacy of cholinomimetic treatment of the Alzheimer-type dementias for especially selected patients warrants further investigation. The participants in the LSRO discussion agreed that precursor administration can compensate for reduced cholinergic activity by stimulating surviving neurons to increased activity. However, as degeneration of cholinergic neurons continues with progression of the disease, the effectiveness of the treatment diminishes. It should be noted that, unlike precursor therapy, physostigmine and arecoline have reliably enhanced the memory functions of patients with Alzheimer's disease (Christie et al., 1981; Davis et al., 1978). Informal reports from a large number of investigators suggest that combinations of drugs may offer a better treatment for Alzheimer's disease than choline or lecithin alone.

Mantione et al. (1981) have developed a potential animal model of Alzheimer-type disease by intracerebroventricular injections of a choline analog, ethylcholine mustard aziridinium, into
mice. The compound produced a long-lasting reduction in the number of sodium-dependent, high-affinity choline transport sites and hence a long-term neurochemical deficit at the cholinergic nerve terminals. A regional decrease in acetylcholine levels in the cortex with no change in the striatum occurred within 3 d. The effects resembled the pattern of presynaptic cholinergic neurochemical deterioration observed postmortem in Alzheimer-type patients.

4. Mania and depression

Janowsky et al. (1972) proposed that mania is the result of adrenergic dominance in the neural substrate whereas cholinergic dominance will lead to depression. As observed by Harris et al. (1979) physostigmine induces depressed moods of sadness, worthlessness, futility, hopelessness, and uselessness in subjects with preexisting affective disorders or unbalanced states. This was interpreted as overcompensation of the acetylcholine-biogenic amine transmitter balance. One consequence of the hypothesis is that physostigmine should be useful in therapy of mania by raising functional acetylcholine which would counteract the hyperfunctional norepinephrine effect. Choline and lecithin should have the same effect.

Physostigmine in proper dosage may produce transient reversal of the symptoms of mania. Janowsky et al. (1973) administered up to 3 mg of physostigmine to eight manic patients in a double-blind test. All exhibited decreased manic symptoms within minutes after the injection; some were depressed. Davis et al. (1978) gave 4 mg of physostigmine to nine patients with mania and obtained significant diminution of the symptoms in the six who were predominantly euphoric manics. One patient who received 20 g/d of choline chloride demonstrated less psychopathology by two tests during treatment and had a reversion at its cessation.

Cohen et al. (1980) have reported a pilot study in which eight patients with manic-depressive illness were treated with phosphatidylcholine derived from soybean lecithin plus lithium and/or neuroleptics. The subjects received a dose of 15 g/d the first week and 30 g/d the second. Three of four subjects receiving a 50% phosphatidylcholine preparation developed vomiting, diarrhea, or motor restlessness on the 5th-7th d of the trials. The manifestations subsided in all three subjects when the phosphatidylcholine was stopped. During the trial one patient improved remarkably in manic symptoms, two improved slightly, and one worsened. Four subjects receiving 90% phosphatidylcholine had no side-effects on 30 g/d plus lithium and/or neuroleptics. All four subjects showed marked improvement while they were receiving the phosphatidylcholine but three had a recurrence of manic symptoms 3-7 d after treatment. The results were thought to warrant controlled trials of phosphatidylcholine in mania.
Hanin et al. (1980) found the profile of erythrocyte choline levels showed wide variability among 78 patients with depression although the plasma choline was in the range of the control subjects. The investigators suggested the wide range of variability in erythrocyte choline reflected endogenous, individually-controlled, predetermined values that related to the integral status of the cell membrane and conceivably could serve as a marker for a specific category of depression.

Jope (1979) reported that chronic lithium treatment stimulated the synthesis of brain acetylcholine in rats. The high-affinity transport of choline and its conversion to acetylcholine were activated to 131% of controls in synaptosomes of rats which had been treated with lithium chloride for 10 d. Millington et al. (1979) found chronic treatment of rats with lithium enhanced the effects of exogenous choline on brain acetylcholine levels presumably by preferentially inhibiting the efflux of choline. Neither acute nor chronic lithium administration changed brain choline levels but did enhance uptake of orally administered choline.

Investigators from several laboratories have reported that chronic lithium administration to patients results in elevations of erythrocyte choline levels (up to 40-110 fold normal values) (Hanin et al., 1981; Jope et al., 1980). The results of these studies favor the interpretation that lithium inhibits the transport of choline across the cell membrane with concurrent breakdown of phospholipid resulting in a net accumulation of free choline within the cell (Hanin et al., 1981). The mechanism of this effect has been demonstrated by Jenden et al. (1981) by a study of kinetics of isotopic exchange of choline between human erythrocytes and a lithium-free incubation medium over a 4-h period. A steady state exchange was verified with a substantial net efflux of choline which represented choline released from phospholipids of the cells.

A recent report suggests that lithium may function by reducing the number of nerve cell surface receptors (Pestronk and Drachman, 1980). Lithium chloride was injected intraperitoneally twice daily into female rats for 1-2 d. Four to seven days after it was denervated by avulsion of the sciatic nerve, the soleus muscle was removed. Extrajunctional acetylcholine receptors, which increase after denervation, were measured by binding of a bungarotoxin. The number of receptors in lithium-treated animals was only 33% that of the saline-injected controls. The investigators suggested that the effects of lithium in reducing the number of acetylcholine receptors in muscle, similar to its ability to reduce dopamine receptors in the brain, may be related to its ability to repress manic-depressive disorders.

In the opinion of some LSRO consultants, the use of choline for treatment of mania may be important as an alternative or as an adjunct to certain drug therapies, for example, lecithin in conjunction with lithium.
5. Other disorders

a. Gilles de la Tourette's disease refers to a syndrome of movement disorders characterized by abrupt involuntary contractions of the muscles of face, neck, trunk, extremities, and respiratory tract (Golden, 1978). The condition is partially responsive to dopamine blockers and may involve altered neuronal receptor site sensitivity (Snyder et al., 1970).

Hanin et al. (1979) found the plasma choline within normal limits but the red cell choline in 20 patients with Tourette's disease was greatly elevated. The significance of this finding is unknown. However, in recent studies conducted in conjunction with Comings, Hanin (1981) concluded elevations in red blood cell choline appear to be genetically transmitted since similar elevations were found in biological relatives. Stahl and Berger (1980) reported successful treatment of six young patients with 0.05 mg/kg of i.v. physostigmine. There were dramatic reductions in tic frequency and vocalizations. It is recognized that not all patients are equally benefited by physostigmine treatment (Tarsy et al., 1974) suggesting similar limitations in the use of choline or lecithin.

Three patients with Gilles de la Tourette's disease were given 10 g/d of choline chloride orally for approximately 4 mo (Barbeau, 1978a). One patient showed some improvement. Two years later Barbeau (1980) reported marked improvement in the same three patients who were started on haloperidol and then changed to a maintenance dose of 40 g/d of lecithin (20% phosphatidylcholine) for 18+ mo. Two of the patients had nearly an abolition of all tics and the third showed a 60% improvement. All showed improved mental abilities. Barbeau (1979) reported an earlier trial with two patients who were given 33 g/d of lecithin (20% phosphatidylcholine) for 17 wk. No improvement was noted in these patients. Polinsky et al. (1980) treated six patients with 45 g/d of lecithin (55% phosphatidylcholine) for periods up to 4 wk. A variety of individual responses was observed, but there was no discernible group benefit.

b. Friedreich's ataxia is a recessive hereditary anomaly resulting in chronic degeneration in spinal cord, cerebellum, and peripheral nerves. Patients exhibit weakness in the legs, tremors in the upper extremities, and abnormal speech. Barbeau (1978a) gave 10 g/d of choline chloride to six ataxic patients. A preliminary assessment of the data suggested subjective improvement in all patients. In another study (Barbeau, 1979), 18.6 g/d (7.2-49 g/d) of a lecithin preparation (20% phosphatidylcholine) were given to ten patients for 23.9 wk. The mean performance score was 35% improved but no control group was used. Two of the patients maintained their improved score during 9 mo on 30 g/d of lecithin. The improvement, however, was insufficient to
change the lifestyle of the patients. There is at this time no convincing evidence that choline, lecithin, and/or physostigmine improve this condition.

c. Six cases of spastic spinocerebellar degeneration (Pierre-Marie type) were not improved by the same regimen of lecithin (Barbeau, 1978b).

d. Ten patients with Parkinson's disease exhibiting severe levodopa-induced dyskinesia were given the same treatment as above (Barbeau, 1979). The lecithin produced a marked reduction in the number and severity of the dyskinesias but at the same time some rigidity and akinesia returned, thus reducing motor performance. This was to be expected if the dyskinesia and parkinsonism represent opposing disruptions of the cholinergic-dopaminergic balance. A number of patients have been treated with deanol on the assumption that it increases cholinergic function. The results have been inconsistent. Miller (1974) reported complete abatement of symptoms in some patients but Klawans et al. (1975) and Laterre and Fortemps (1975) considered the compound ineffective in combatting the dyskinesia.

e. Papavasiliou and Rosal (1979) treated seven patients with levodopa-induced dyskinesia with choline chloride and L-α-methyldopa hydrazine according to a double-blind protocol. The compounds were administered in six portions daily. Choline chloride dosage was gradually increased to a maximum of 200-300 mg/kg/d body weight. α-Methyldopa hydrazine was given to counteract peripheral cholinergic effects. One subject had transitory vomiting, three excessive salivation, and three flatulence. Clinical evaluation continued four times daily over the 25-45 d trials. Two patients had diminution of levodopa-induced dyskinesia and another exhibited an increase in parkinsonian signs. Two became more withdrawn and depressed during choline administration. These investigators proposed that an optimum balance between the opposing dopaminergic and cholinergic effects is essential for management of such patients.

f. The myasthenic syndrome is considered to be a prejunction disorder which results from a defect in transmitter release. It is a rare disorder sometimes associated with bronchiogenic carcinoma (Elmqvist and Lambert, 1968). The principal symptoms are weakness and easy fatigability of proximal muscles of limbs.

One myasthenic patient was characterized by depression and facilitation (Kranz et al., 1980). Choline bitartrate was given i.v. at a rate of 27 mg/min choline for 70 min for a total
dose of 38 mg/kg. The patient exhibited clinical features consistent with increased cholinergic autonomic stimulation. Then choline chloride was given orally (210 mg/kg/d) for 4 wk. The treatment increased muscle action potential amplitude in response to single shocks at 1-20 Hz. The significance of the results in terms of choline or lecithin treatment of the condition awaits further investigations.
V. OTHER METABOLIC EFFECTS OF ORAL ADMINISTRATION

A. CARDIOVASCULAR ASPECTS

1. Lipotropic effect

It has long been held that polyunsaturated fats in the diet can reduce hypertriglyceridemia and hypercholesterolemia and thus reduce the risk of heart disease (National Research Council, 1958; Vergroesen, 1977). The assumed causal relationship between hypertriglyceridemia and coronary heart disease, however, has been questioned recently (Hulley et al., 1980). The effects observed with unsaturated fat diets have been related to their high linoleic acid content. Also, choline as well as some of its analogs exerts a lipotropic effect (Griffith and Nyc, 1971). To exploit the lipotropic effects of choline as well as linoleic acid, soybean lecithin and phosphatidylcholine have been tested for the treatment of the hyperlipidemias.

To show the importance of unsaturated fatty acids in lecithin, Rosseneu et al. (1979) placed four chimpanzees on the following diets successively for 1 mo each: control, enriched with phosphatidylcholine high in linoleic acid, and enriched with phosphatidylcholine containing only saturated fatty acids. The unsaturated diet produced a striking increase in the esterification of cholesterol and lysolecithin content of HDL₃ with an accompanying decrease in VLDL and plasma triglycerides. On the other hand, the saturated phosphatidylcholine diet had opposite effects which were considered likely to enhance the progression of atherosclerosis.

Hyperlipemic rhesus monkeys were given 400 mg/kg/d of phosphatidylcholine for 6 wk (Nicolosi et al., 1979). The responses were variable but there were significant reductions in plasma cholesterol and triglycerides. The phosphatidylcholine feeding did not change plasma phospholipid levels or lecithin-cholesterol acyltransferase activity.

Similar results were obtained by Wong et al. (1980). Seven rhesus monkeys were hyperlipidemic as a result of eating a semi-purified diet containing sucrose, corn oil casein, and 4 g/kg of cholesterol for 10 yr. When the diet was supplemented with 17 g/kg of phosphatidylcholine, there was a significant decrease in plasma triglycerides and cholesterol followed by a rebound when the supplement was omitted. Likewise, the phosphatidylcholine supplement decreased LDL- and increased HDL-cholesterol concentrations. Since corn oil was in the diet the authors concluded changes in triglycerides and cholesterol were due to the phosphatidylcholine fed rather than the polyunsaturated fat.
When pure phosphatidylethanolamine in saline was fed to rats, the plasma phospholipids were only slightly increased, while the total phospholipids in liver were greatly reduced (Maclagan et al., 1966). Feeding phosphatidylethanolamine in olive oil caused a reduction of liver phospholipids, but only in the phosphatidylycholine fraction. Lecithin, when fed, did not produce significant changes in liver, plasma, or tissue phospholipid levels. These results suggested that liver phospholipid synthesis was depressed by feeding phosphatidylethanolamine.

For at least four decades soybean lecithin has been administered to patients as a rich source of linoleic acid (Select Committee on GRAS Substances, 1979). Interpretation of studies on the reduction of blood lipids by administration of lecithin or phosphatidylcholine has been controversial. ter Welle et al. (1974) found no changes in plasma triglyceride levels in 12 subjects given 1.2 g/d of soybean phosphatidylcholine for 10-12 wk. The authors cited other investigations which agree with their findings but contrary evidence also has accumulated as described below.

Oral administration of 1.7 g of Lipostabil® daily for 5 wk to five hyperlipoproteinemic males caused a 16% reduction in their serum triglycerides and an increase of \(\alpha\)-lipoproteins (HDL) containing linoleic acid (Svanberg et al., 1974). Cobb et al. (1980) found a small but significant reduction in plasma triglycerides and total phospholipid levels in seven volunteers who ingested 22.5 g of soybean lecithin daily for 4 wk.

Hypertriglyceridemia induced by a high sucrose intake was significantly decreased in male subjects by feeding 3 g/d of phosphatidylcholine for 3 wk (Ditschuneit et al., 1976). The ratio of polyunsaturated to saturated fatty acids increased in the plasma lipoproteins. This was attributed to the stimulating effect of phosphatidylcholine on lipoprotein lipase.

Blaton et al. (1976) found that i.v. therapy with 1.0 g/d of phosphatidylcholine for 14 d followed by ingestion of 1.8 g of "Lipostabil Forte®" (phosphatidylcholine in capsules) for 106 d by 69 patients produced a decrease in plasma lipoproteins that was significant, but less than obtained during initial i.v. therapy.

* A soybean lecithin preparation dispersed with deoxycholic acid in a 1:1 ratio, and containing 84% phosphatidylcholine, 2% lyso-phosphatidylcholine, 13% phosphatidylethanolamine, and 1% lyso-phosphatidylethanolamine in the presence of 10% triglycerides and a total equivalence of 65% linoleic acid (Blaton et al., 1976).
2. Lipoproteins and hypercholesterolemia

One of the important functions of phospholipids is their formation of lipoproteins which transport cholesterol. In recent years considerable advances have been made in understanding the structure and function of these blood components (Hamilton, 1978; Havel, 1980; Tall and Small, 1978). In the presence of dietary triglycerides, chylomicrons containing about 9% phospholipid and 2% protein are released into the lymph from the small intestine and VLDL are released from the liver. Chylomicrons and VLDL are reduced to remnants by lipoprotein lipases. The VLDL, in losing triglycerides, become more dense and have a transient existence as intermediate-density lipoproteins (IDL). IDL are converted by the liver to more stable LDL in which the phospholipid has increased to about 20% and triglycerides have decreased to 10% of the particle. The LDL are characterized by a core enriched in cholesterol and cholesterol esters (8% and 37% respectively). LDL transport these to tissue receptors and to a minor extent to the liver.

HDL are produced principally by the liver and possibly by the small intestine (Tall and Small, 1978). As found in the plasma their contents are variable but contain about 24% phospholipid, 15% cholesterol esters, and only 4% triglycerides. Formation of HDL is thought to cause a redistribution of cholesterol among the plasma lipoproteins (Carew et al., 1976) and provide a transport vehicle for the excretion of cholesterol into the bile (Hamilton, 1978). The action of lecithin-cholesterol acyltransferase probably plays an essential role in HDL-cholesterol transport (Steinberg, 1979). Transfer of cholesteryl esters from HDL to VLDL and LDL provides a pathway for transport to tissues (Havel, 1980).

The importance of cholesterol intake and the resulting hypercholesterolemia on the genesis of heart disease has long been a matter of controversy (Keys, 1980; National Dairy Council, 1979; Tan et al., 1980). From considerations of his 25-yr study of adult men, Keys (1980) suggested that there is presently an overemphasis on the relationships of serum HDL-cholesterol to the development of heart disease. As noted above, there is a general consensus that substitution of polyunsaturated fats for saturated in the diet lowers plasma cholesterol and triglyceride concentrations in man. Since the decrease in serum cholesterol was related to the polyunsaturated fatty acid content of the diet (Vergroesen, 1972), corn oil was considered superior to phosphatidylcholine as a source of linoleic acid.

More recently the reduction of total serum cholesterol has been ascribed primarily to decreases in the LDL-cholesterol (Steinberg, 1979). Compensating effects in other lipid fractions probably account for the contrasting results obtained on serum cholesterol in other investigations.
Administration of large amounts of phosphatidylcholine alters the lipoprotein pattern of the plasma temporarily, reducing chylomicron size and numbers and lowering the cholesterol content (Beil and Grundy, 1980). The effect is dose-related, the greatest reductions being obtained with sustained administration of 25 g/d or more of lecithin. The investigators found that a high intake of phosphatidylcholine (67% linoleic acid) influenced the size and density of chylomicrons and VLDL. Phosphatidylcholine infused duodenally at the rate of 9 g/h for 10 h into human subjects produced triglyceride-rich VLDL predominantly, in contrast to safflower oil (6 g/h) which formed mainly chylomicrons. Another effect of the high-phospholipid intake was production of small chylomicrons with a high phospholipid (coat) to triglyceride (core) ratio. Phosphatidylcholine infusion decreased cholesterol absorption from the upper part of the intestine.

Jenkins et al. (1981) found HDL plasma levels in weanling male rats to be related to the source of the protein in their diet. At 10% protein content, lactalbumin was more effective than collagen, soybean protein concentrate, or wheat gluten. Soybean lecithin (53% phosphatidylcholine and 63% linoleic acid) was added to the diet at 2.5 and 5% levels. Soy protein concentrate- and lactalbumin-fed rats had an increased plasma cholesterol, liver nitrogen, and liver phospholipid content. With increase in lecithin intake there was a significant increase in liver phospholipid ratio for all sources except wheat gluten.

The effects of polyunsaturated fats on plasma and tissue cholesterol levels have led to studies employing high intakes of soybean lecithin, which contains large amounts of linoleic acid residues. Further justifications for administration of soybean lecithin for preventing and ameliorating hypercholesterolemia and atherosclerosis are based on the lipotropic effect of lecithin on fatty livers; the solubilizing effects of lecithin on cholesterol, and the decreased sclerogenic activity when cholesterol was esterified with linoleic or arachidonic acid (Krumdieck and Butterworth, 1974). Esterification of cholesterol is mediated by lecithin-cholesterol acyltransferase, which shows high activity with phosphatidylcholines possessing linoleic or arachidonic acid in the 2-position. The presence of larger amounts of saturated fatty acids in egg yolk lecithin was considered to contribute a hypercholesterolemic effect (Adams et al., 1967).

Kesten and Silbowitz (1942) fed soybean lecithin to rabbits maintained on an atherogenic diet. Hypercholesterolemia and arteriosclerosis were reduced in five of seven animals. This effect was attributed to phosphatidylcholine because choline itself had been reported previously to have no effect on the reduction of hypercholesterolemia in rabbits. Kritchevsky et al. (1979) fed rabbits an atherogenic diet for 6 mo and then supplemented it with 1% egg yolk which provided 350-465 mg/kg body weight lecithin. The lecithin had no effect on the course of atherosclerosis but decreased liver triglyceride levels 27%. Liver cholesterol and phospholipids were increased.
Adlersberg and Sobotka (1943) reported striking decreases in serum cholesterol levels in five patients receiving 12-15 g of natural soybean lecithin in the diet for 2-3 mo. Six mo after the lecithin addition was discontinued, the cholesterol concentrations had returned to their former high levels. Similar results were obtained by Steiner and Domanski (1944) who administered 25 g of soybean lecithin in the diets of eight patients for 6 wk. Of 15 hypercholesterolemic patients studied by Morrison (1958), 12 showed an average 41% reduction of serum cholesterol levels after taking 36 g/d of refined soybean lecithin for 3 mo.

Davis et al. (1965) studied 362 hypercholesterolemic patients who were maintained on their usual diet supplemented with 25 g/d of soybean lecithin for periods of 6-18 wk. Plasma cholesterols were significantly reduced in 6 wk and were maintained in 192 patients who were observed after 18 wk. Six weeks after the regimen was stopped a rise in cholesterol was noted. Skořepa et al. (1976) reported that the ingestion of 1.8 g/d of phosphatidylcholine for 8 wk reduced total serum cholesterol by 12% in 12 hypercholesterolemic patients; the polyunsaturated acids in cholesterol esters increased.

On the other hand, ter Welle et al. (1974) were unable to demonstrate changes in the total cholesterol, triglycerides, phospholipids, and total lipids in the lipoprotein fractions, and the weight percentage of linoleic acid in serum cholesterol esters after administering 1.2 g/d of soybean lecithin (85% phosphatidylcholine) for 10-20 wk to 12 patients with type II hyperlipoproteinemia. After the first experimental period, the dose was raised to 2.4 g/d for 4 mo. The negative results were in agreement with the findings of Davies and Murdoch (1959), Butler et al. (1960), and Svanberg et al. (1974), all of whom used Lipostabil® as a source of lecithin. Failure of the treatment in such studies was ascribed by Simons et al. (1977) to the low dosage of lecithin used. However, Cobb et al. (1980) found human subjects ingesting 22.5 g of soybean lecithin daily for 4 wk had no changes in plasma total cholesterol or cholesterol esterification. Simons (1978) and Simons et al. (1977) decreased plasma cholesterol significantly in three of seven patients with type IIa or IIb hyperlipoproteinemia by treatment with 20-30 g/d of phosphatidylcholine (56% linoleic acid) for 6-36 wk. The authors concluded that the variable results from lecithin feeding observed by others generally resulted from enhanced tissue mobilization and excretion of cholesterol with possibly a compensatory increase in endogenous cholesterol synthesis. The balance of these phenomena would govern any subsequent decrease in plasma cholesterol.

A special effect of lecithin on hypercholesterolemia was found in a recent study. When refined soybean lecithin or corn oil was fed for two 2-mo periods to twelve normolipidemic and six hypercholesterolemic subjects in amounts varying from 5-36 g/d, there was a slight but significant increase in HDL-cholesterol.
accompanied by a decrease in LDL-cholesterol (Childs et al., 1981). This was attributed to a mechanism independent of the polyunsaturated fatty acid content of the lecithin because no such effect was observed with corn oil. A similar conclusion was reached by Wong et al. (1980) from feeding phosphatidylcholine to hyperlipidemic monkeys. Both corn oil and lecithin reduced LDL-cholesterol levels.

3. Atherosclerosis

Accumulation of cholesterol and its esters in the arterial wall is enhanced by the presence of cholesteryl olate, and cholesteryl palmitate which are resistant to hydrolysis by a cholesteryl esterase. Exchange of these esters with linoleic acid mediated by lecithin-cholesterol acyltransferase renders them more susceptible to hydrolysis (Patelski, 1976). According to Howard and Patelski (1976), the antiatherogenic effect of polyenoic phosphatidylcholine thus results from an increase in cholesteryl ester hydrolysis and a decrease in cholesteryl ester synthesis (acyltransferase activity). Samochowiec (1976) extended these observations to oral administration of phosphatidylcholine. Rats and miniature pigs were placed on an atherogenic diet for 60 d. Graded doses of polyenoic phosphatidylcholine, given by gavage, (1900 mg/kg/d and 90 mg/kg/d, administered to rats and miniature pigs, respectively) produced significant regressive and preventive effects on atherogenesis. The total serum lipids decreased slowly over the 60-d period but a change in the fatty acids toward more unsaturation was observed in a few days. Doses of 2800 mg/kg/d in rats significantly reduced the development of atherosclerosis.

Stafford and Day (1975) reported that, of all the phosphatides tested, only phosphatidylcholine produced regression of experimentally-induced atherosclerosis in Japanese quail and the effect was limited to polyenoic lecithins. Horsch et al. (1976) observed that phospholipid synthesis from $^{14}$C-acetate in rabbits was significantly depressed by i.v. polyenoic phosphatidylcholine but there was no change in the arterial lipid composition although the linoleic-oleic acid ratio increased in serum phospholipids and cholesterol esters. The oral administration of 1800 mg/d of phosphatidylcholine for 30 d to 20 patients with circulatory disturbances increased muscle and cerebral blood flow in extremities probably as a result of a decrease in blood viscosity (Klemm, 1976). The hematocrit and plasma or blood viscosity in eleven healthy test subjects and eight patients with chronic arterial occlusive disease were not affected 45 min after i.v. injection of phosphatidylcholine. Ehrly and Blendin (1976) concluded the increased blood flow must have been due to a more protracted change in the composition of the blood vessels. However, the passage of erythrocytes through a capillary was improved 25%, probably reflecting an increased deformability of the cells by alteration of the membrane phosphatides.
Schneider et al. (1976) gave 3 g/d of phosphatidylcholine for 3.5 mo to 31 patients suffering from hyperlipoproteinemia. By the eighth week a decrease in the aggregability of the platelets was observed. This may have resulted from an increased synthesis of prostaglandin E₁ or simply from alteration of the membrane characteristics of the platelets.
VI. STUDY CONCLUSIONS

NOMENCLATURE, COMPOSITION, PURITY

- Use of the terms, "lecithin" and "phosphatidylcholine" interchangeably in the public media and scientific literature has created confusion. Commercially available lecithins are mixtures of phosphatides containing as little as 20% phosphatidylcholine. Use of the trivial name, lecithin, in the scientific literature should be restricted to commercial products and be accompanied by data on composition where available. It should not refer to phosphatidylcholine.

- Specifications for food grade lecithin presently do not identify ranges of therapeutically important constituents such as phosphatidylcholine and linoleic acid. The specificity and accuracy of methods presently available for determining phosphatidylcholine and other phosphatides should be used to full capacity. Minor ingredients of commercial lecithin may have physiological effects that should be recognized.

- Clinical experience has demonstrated an urgent need for a chemically-defined supply of phosphatidylcholine of known purity for use in clinical research. The phosphatidylcholine content should be at least 90-95% with accompanying information on other components such as phosphatides, sugars, heavy metals, pesticides, and optical isomers. In addition, it would be useful to identify fatty acid distribution and have additional information on toxicity when appropriate.

SUPPLEMENTAL CHOLINE AND LECITHIN

- Available evidence indicates that the North American diet supplies the average adult with 1-5 g/d of lecithin. With special selection, total choline in the food may amount to 500-900 mg/d. Dietary phosphatides induce variations in plasma composition which, in turn, influence the distribution of phosphatides in membranes and lipoproteins. Digestion, degradation, and resynthesis tend to maintain structural phospholipids in optimal form for physiological functions. There is little basis for ascribing benefits or hazards to healthy persons from supplementation of their diets with lecithin or choline.

- The intrinsic toxicity of lecithin is low. Oral administration is self-limiting since ingestion of large amounts of commercially-available lecithin produces acute gastrointestinal distress. There is little likelihood that individuals will undertake long-term consumption of amounts of 25 g or more per day.
Choline salts are not well accepted by patients because of an objectionable body odor resulting from the intestinal production of trimethylamine. Additional peripheral cholinergic effects of large doses of choline, lecithin, or phosphatidylcholine are reduction in appetite, vomiting, bloating, diarrhea, and behavioral effects including depression or aggressive tendencies. The side-effects from phosphatidylcholine are notably less severe than observed from equivalent doses of lecithin.

TISSUE LEVELS AND STORES

Exogenous choline and lecithin contribute to membrane phospholipids more extensively than to synaptic acetylcholine; they probably contribute to acetylcholine of non-nervous tissues and of non-cholinergic neurons.

The cholinergic system can be manipulated within limits by use of compounds that increase the choline content of the plasma and subsequently the brain. However, the dose-response characteristics of choline and lecithin as precursors of synaptic acetylcholine are known incompletely. In experimental animals, it is difficult to increase brain acetylcholine more than 30-50%. Variations in experimental results have led to disagreements among investigators on the ability of choline and lecithin to cause increased cholinergic function and/or acetylcholine turnover.

Although most investigators accept the conclusion that increased choline or lecithin intakes raise brain acetylcholine, some propose that high brain levels of choline do not directly contribute to an increase of brain acetylcholine synthesis. Alternately, the cholinomimetic effect may be enhancement of release of bound acetylcholine or a direct agonist effect of choline. Choline is a weak agonist at synaptic sites such as in neuromuscular junctions, ganglia, the adrenal medulla, and the cerebral cortex. The cholinomimetic effect of choline may be more important in acetylcholine-depleted receptors than in normal.

Placental transport of choline produces a concentration gradient in the fetus. The effects of inducing higher levels of choline in the fetus, if possible, are unknown. However, the level of choline in the plasma of the newborn is higher than that of infants, and its function is incompletely understood.
MEMORY, AGING, NEUROLOGICAL DISORDERS

- Animal studies suggest that stimulation of memory by oral lecithin or choline should be successful. However, preliminary studies indicate that these compounds alone have not improved memory in human subjects.

- There are no scientific data to support the hypothesis that lecithin ingestion will retard aging in the brain although the effects on memory may be ameliorated somewhat by cholinomimetics.

- Choline and lecithin administration have produced improvements in some patients with tardive dyskinesia. Clinical studies have resulted in benefits to a limited number of patients with Gilles de la Tourette's disease, Friedreich's ataxia, levodopa dyskinesia, and Alzheimer's disease. Results with Huntington's disease have not been encouraging. Other conditions such as spastic spinocerebellar degeneration, mania, and myasthenic syndrome have been studied in few patients. Precursor therapy of neurologic disorders with lecithin or choline produces no permanent cures; that is symptoms recur after discontinuance of the treatment. More trials are necessary to evaluate possible beneficial effects of choline and lecithin.

- A long-term effect of lecithin therapy could be pseudo-parkinsonism resulting from a cholinergic-dopaminergic imbalance; withdrawal of high lecithin intake might produce similar effects. Other adverse effects such as those on drug tolerance and sensory processes are possible but have not been observed. The onset of depression has been observed in a number of clinical trials. Cholinergic agonists may cause depression of motor activity.

- The stimulative effect of increased acetylcholine in the central nervous system may be limited by the number of responsive neurons in the brain. Extensive degeneration of both pre- and postsynaptic elements may obviate the use of cholinomimetic substances. For patients with organic neurologic disorders, the effective dose will have to be determined individually. Alternatively, direct acting cholinomimetics may be desirable.

LIPOTROPIC EFFECT

- The lipotropic action of lecithin is due to its polyenoic fatty acids and phosphatidylcholine. Incomplete data suggesting that lecithin is superior to unsaturated fats and oils in treatment of hypercholesterolemia and atherosclerosis have been collected from both animal and human studies, but, as yet, are equivocal.
VII. SUGGESTIONS FOR FUTURE CONSIDERATION

The ad hoc group discussions and the review of pertinent literature identified a number of considerations for future research.

NOMENCLATURE, COMPOSITION, PURITY

- The recommendations of an ad hoc committee on nomenclature and methodology (Hanin, 1979) provide a model for specifications that are needed for lecithin. Commercially available supplies of food grade lecithin should be analyzed and labeled to indicate:

1. percentage composition of phosphatidylcholine, total choline, free choline, free ethanolamine, free serine, phosphatidylethanolamine, lysophosphatidylcholine, phosphorylcholine, and total phosphatide content;

2. fatty acid pattern and composition of the preparation. Any fatty acid comprising more than 5% of the total should be identified;

3. heavy metal and pesticide content;

4. volatile residues; and,

5. residual peroxide content.

- The following information should also be provided for lecithin products which are to be used for investigational purposes: tissue source, solubility, procedures used in the preparation, documentation of acceptability for human use, and any adverse effects which have previously been observed in use of the product. Lecithin producers are urged to conduct multigenerational, high-dose studies on the effects of lecithin on experimental animals.

- Increasing use of phosphatidylcholine as a dietary supplement may stimulate commercial production of the racemic form. It is anticipated that the unnatural form may have lesser or no physiological activity in enzyme-catalyzed reactions, but may have some yet undetermined toxic effects. Conversely, if sn-1-phosphatidylcholine is nontoxic it would provide an ideal placebo which is currently not available for controlled studies. Further research into these possibilities will be necessary should the racemic form become commercially available as a dietary supplement.
There should be a concerted effort to develop a large stable commercial supply of phosphatidylcholine of 90-95% purity for clinical trials. A specification for the fatty acid composition should be developed. Supplies of phosphatidylcholine isotopically labeled in the choline moiety should be made available for investigational use.

TISSUE LEVELS AND STORES

Present knowledge of the effects of each of the phosphatides on membranes should be extended. The theoretical aspects of choline uptake and utilization by the nervous system need further clarification as a means for developing new clinical approaches to therapy including measures for prevention of diseases susceptible to preventive measures. Animal models should be sought for such diseases. The effects of choline on acetylcholine turnover in ganglia or neuromyial junctions should be investigated. Correlations between neurologic functions and plasma choline concentrations should be sought.

Information on effects of supplemental lecithin on development of the fetus is limited. There is a need for determining if cholinomimetic agonists affect normal growth and development.

MEMORY, AGING, NEUROLOGICAL DISORDERS

There is a need to clarify the effects of such factors as aging, anoxia, radiation, and limitations of metabolizable energy sources on the cholinergic system. Determination of the loci of action of phosphatidylcholine on cholinergic functions in various tissues and factors influencing specific functions in such tissues should be investigated.

Despite the attendant difficulties and expense involved, considerably more clinical trials are required to determine the effectiveness of choline and lecithin or phosphatidylcholine for treatment of motor disturbances, memory loss, and mental illnesses. The implications of high red blood cell choline levels in Gilles de la Tourette's disease including possible genetic factors are intriguing and need further investigation. This might be considered in conjunction with the elevated choline levels that have been observed in mania and with the effect of lithium in elevating erythrocyte choline levels.
LIPOTRIPC EFFECTS

* The effects of linoleic acid upon prostaglandin and prostacyclin metabolism and the physiologic and pharmacologic effects should be fully investigated.

* Additional clinical trials are required to determine the effectiveness of choline and lecithin for treatment of hypercholesterolemia and atherosclerosis. The relative effectiveness of phosphatidylcholine and corn oil should be evaluated further.
VIII. LITERATURE CITED


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IX. STUDY PARTICIPANTS

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X. APPENDIX

A. PROPERTIES OF CHOLINE

1. Nature

Choline, 2-hydroxyethyltrimethylammonium hydroxide, is a natural constituent of foods occurring principally as a component of phosphatidylcholine, sphingomyelin, and other related compounds (Griffith and Nyc, 1971*). The free base is hygroscopic but can be obtained as a colorless crystalline mass by drying in a high vacuum. Because of its instability in air the compound is marketed as the chloride or bitartrate.

2. Uses

The bitartrate or the chloride is added to infant formulas at about the level of 0.1% of the dry powder. Special dietary products for adults sometimes have choline salts added. It has been estimated that an adult may receive as much as 17 mg/d of choline from such foods (Select Committee on GRAS Substances, 1975).

3. Specifications

Food Chemicals Codex states that food grade choline chloride or choline bitartrate should assay at least 98% calculated on the anhydrous basis. Impurity limits are arsenic, 3 ppm; lead, 10 ppm; and heavy metals (as lead), 20 ppm (National Research Council, 1981a).

B. PROPERTIES OF LECITHIN

1. Nature

Natural lecithin varies in color from a light tan to a dark reddish-brown depending upon the source and subsequent exposure to light and air. It is unstable to light and air, rapidly undergoing oxidation and polymerization. The color may be diminished by bleaching with hydrogen peroxide. The consistency varies from a fluid to a plastic solid. Plastic and fluid forms may be produced either as unbleached, single-bleached, or double-bleached grades, resulting in six types of natural lecithin (Sullivan and Szuhaj, 1975).

* References cited are found on pages 67-93.
2. **Nomenclature**

The commercial use of the term lecithin is at variance with the scientific literature. In the biomedical sciences lecithin is a trivial term which refers specifically to phosphatidylcholine, 1,2-diacyl-sn-glycero-3-phosphocholine, and the term, cephalin, is employed for phosphatidylethanolamine. Many research reports in which lecithin is used for its phosphatidylcholine content, or for its linoleic acid content, fail to give the percentage composition of the active component. Because industry has firmly established the term, lecithin, to refer to the mixture of phosphatides obtained from soybean or other oils, it would be clarifying if scientific investigators would abandon its use when referring specifically to phosphatidylcholine.

3. **Composition**

In commercial usage, lecithin refers to a crude or refined phospholipid mixture obtained as a by-product of the refining of vegetable oils (Van Nieuwenhuyzen, 1976) (Table A-1). Although soybean oil is the present commercial source, crude corn oil, safflower oil, cottonseed oil, or torula yeast are other potential food sources of commercial food grade lecithin in the United States. In other countries, commercial lecithins may be prepared also from rapeseed or peanut oil. Egg lecithin has been prepared for scientific purposes. Generally, food grade lecithin is isolated by hydration of solvent-extracted soybean oil. Lecithin separates as a sludge and is isolated as a gum which is dried to a moisture content of about 1%. This lecithin is termed "natural" (National Soybean Processors Association, 1980). Natural soybean lecithin contains up to 35% soybean oil; removal of 90-95% of the oil yields an "oil-free" product termed "refined" lecithin. Depending upon the method of extraction, refined lecithins may be termed oil-free, alcohol-soluble, alcohol-insoluble, or custom blended (Sullivan and Szuhaj, 1975). A third and distinct type of lecithin includes chemically-modified products. The principal modification is hydroxylation which improves water dispersion and emulsifying properties.

Commercial lecithin is available for purchase as a dietary supplement in two forms: granules stabilized by calcium phosphate, and capsules containing a dispersion in oil. The product contains a mixture of phosphatides as shown in Tables A-1 and A-2. A number of commercial firms produce refined grades of lecithin, in which the phosphatidylcholine content may be enhanced to 60-95%. Such special grades are principally for research purposes (Wurtman, 1980). Fractions rich in phosphatidylinositol or phosphatidylethanolamine are also available.
Table A-1. Composition of a Commercial Sample of Oil-Free Soybean Lecithin*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Percent by weight†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipids:</td>
<td></td>
</tr>
<tr>
<td>phosphatidylcholine</td>
<td>29.0 ± 2.1</td>
</tr>
<tr>
<td>phosphatidylethanolamine</td>
<td>23.5 ± 0.7</td>
</tr>
<tr>
<td>phosphatidylinositol</td>
<td>15.1 ± 0.8</td>
</tr>
<tr>
<td>phosphatidic acid</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td>unestimated phospholipids§</td>
<td>7.9 ± 1.2</td>
</tr>
<tr>
<td>Total phospholipids</td>
<td>82.5</td>
</tr>
<tr>
<td>Glycolipids:</td>
<td></td>
</tr>
<tr>
<td>esterified steryl glucosides</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>steryl glucosides + cerebrosides</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td>digalactosyl diglyceride</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>unestimated galactolipids§</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>Total glycolipids</td>
<td>15.0</td>
</tr>
<tr>
<td>Neutral lipids:</td>
<td></td>
</tr>
<tr>
<td>triglycerides</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>sterols</td>
<td>0.2 ± 0.07</td>
</tr>
<tr>
<td>free fatty acids</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>unestimated neutral lipids**</td>
<td>0.2 ± 0.15</td>
</tr>
<tr>
<td>Total neutral lipids</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Adapted from Erdahl et al., 1973.
† Mean ± 1 standard deviation.
§ Unestimated phospholipids include acylphosphatidylethanolamine, diphosphatidylglycerol, lysophosphatidylethanolamine, lysophosphatidylcholine, and unknowns.
¶ The unestimated glycolipids were not identified.
** Unestimated neutral lipids include diglycerides, monoglycerides, sterol esters, pigments, and unknowns.
Table A-2. Composition of Lecithins*

A) Phosphatide content of crude vegetable oils

<table>
<thead>
<tr>
<th>Oil</th>
<th>Phosphatide Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>1.1-3.2 (average about 1.8)</td>
</tr>
<tr>
<td>Corn</td>
<td>1-2</td>
</tr>
<tr>
<td>Wheat</td>
<td>0.08-2.0</td>
</tr>
<tr>
<td>Cottonseed</td>
<td>0.7-0.9</td>
</tr>
<tr>
<td>Rice</td>
<td>0.5</td>
</tr>
<tr>
<td>Linseed</td>
<td>0.3</td>
</tr>
<tr>
<td>Peanut</td>
<td>0.3-0.4</td>
</tr>
<tr>
<td>Sesame seed</td>
<td>0.1</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>0.1</td>
</tr>
</tbody>
</table>

B) Fatty acid composition (% by weight) of vegetable phosphatides

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Soybean†</th>
<th>Soybean§</th>
<th>Peanut</th>
<th>Linseed</th>
<th>Sunflower</th>
<th>Cottonseed</th>
<th>Rapeseed†</th>
<th>Rapeseed§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitic</td>
<td>17.3</td>
<td>11.7</td>
<td>16.2</td>
<td>11.3</td>
<td>14.7</td>
<td>17.3</td>
<td>8.3</td>
<td>15.8</td>
</tr>
<tr>
<td>Stearic</td>
<td>4.0</td>
<td>2.8</td>
<td>10.6</td>
<td>5.1</td>
<td>7.3</td>
<td>1.5</td>
<td>2.1</td>
<td>6.3</td>
</tr>
<tr>
<td>C20-C26</td>
<td>1.4</td>
<td>7.1</td>
<td>3.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>2.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>5.5</td>
<td>8.6</td>
<td>3.5</td>
<td>1.5</td>
<td>1.5</td>
<td>2.1</td>
<td>6.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Oleic</td>
<td>19.0</td>
<td>9.8</td>
<td>47.1</td>
<td>33.6</td>
<td>19.3</td>
<td>20.3</td>
<td>22.4</td>
<td>13.0</td>
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<tr>
<td>Linoleic</td>
<td>53.0</td>
<td>63.3</td>
<td>22.7</td>
<td>20.4</td>
<td>45.9</td>
<td>44.4</td>
<td>42.2</td>
<td>62.9</td>
</tr>
<tr>
<td>Linolenic</td>
<td>3.7</td>
<td>4.0</td>
<td>17.4</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C20-C26 (unsat)</td>
<td>1.5</td>
<td>5.5</td>
<td>4.1</td>
<td>3.2</td>
<td>5.5</td>
<td>6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erucic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C) Fatty acid composition (% by weight) of animal phosphatides

<table>
<thead>
<tr>
<th>Phosphatide Origin</th>
<th>Saturated Acids</th>
<th>Unsaturated Acids†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C14</td>
<td>C16</td>
</tr>
<tr>
<td>Liver (pig)</td>
<td>12.1</td>
<td>15.4</td>
</tr>
<tr>
<td>Liver (beef)</td>
<td>1.3</td>
<td>28.2</td>
</tr>
<tr>
<td>Liver (human)</td>
<td>12.6</td>
<td>21.8</td>
</tr>
<tr>
<td>Heart (beef)</td>
<td>14.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Suprarenals (beef)</td>
<td>1.2</td>
<td>23.8</td>
</tr>
<tr>
<td>Brain (human)</td>
<td>8.0</td>
<td>21.0</td>
</tr>
<tr>
<td>Egg (hen)</td>
<td>31.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Milk (cow)</td>
<td>5.3</td>
<td>16.1</td>
</tr>
</tbody>
</table>

* From: Mattil et al., 1964.
† Fraction insoluble in alcohol (cephalins).
§ Fraction soluble in alcohol (lecithins).
¶ The numbers in parentheses refer to the average number of hydrogen atoms required to saturate the fraction under consideration.
** Arachidonic acid mainly.
4. Commercial uses

Industrial uses depend upon emulsification and dispersion properties of the type of lecithin which facilitates dispersion of dyes and pigments in oil, latex paint, varnishes, lacquers, and inks. Emollient properties of lecithin are utilized in cosmetics, shampoos, hair rinses, skin conditioners, and especially soaps. Other applications include the production of magnetic tapes, special plastics, and metals such as aluminum foil (Sullivan and Szuhaj, 1975).

Food grade lecithin is used as an antispattering agent and emulsifier in the margarine industry. Lecithin reduces the amount of cocoa butter needed in chocolate candies and candy coating (Sullivan and Szuhaj, 1975). It improves the moisture tolerance in confections such as caramels, taffy, and chewing gum, and retards fat and sugar crystallization. Lecithin is used in a large variety of dry mixes, including non-dairy coffee whiteners, cake and icing mixes, powdered beverages, instant cereals, and desserts. The commercial baking industry uses lecithin in bread, biscuits, wafers, and pastry products as a dough conditioner, antistaling agent, and finished texture stabilizer. In pastry products, lecithin improves yeast leavening, retards blighting in short dough, and improves the layering and flakiness of puff and Danish pastries. Lecithin is the active ingredient in pan-release agents which are provided for household use. The multiplicity of food uses results in the presence of added lecithin in a wide variety of foods (Table A-3).

In biological and clinical studies, commercial refined lecithin has been utilized either for its choline content or for the presence of polyunsaturated fatty acids. The proportion of linoleic and other polyunsaturated fatty acids in lecithin preparations varies with the plant or animal source. Table A-1 shows the fatty acid distribution in a number of lecithins (Mattil et al., 1964).

The instability of soybean lecithin to oxidation results from its high degree of unsaturation. Hydrogenation to improve shelf stability could cause loss of linoleic and arachidonic acids; and production of trans and positional fatty acid isomers could affect the biological properties of the phosphatides.

5. Specifications

The Food Chemicals Codex (National Research Council, 1981b) describes food grade lecithin as originating from soybeans and consisting of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol in unspecified ratios. Various amounts of other substances such as triglycerides, fatty acids, carbohydrates, carotenoids, amino acids, biotin, riboflavin,
Table A-3. Level of Addition of Lecithin and Lecithin Modified with Hydrogen Peroxide to Foods by Food Category (Subcommittee on Review of the GRAS List--Phase II, 1972)

<table>
<thead>
<tr>
<th>Food Category</th>
<th>Lecithin Weighted mean percent</th>
<th>Lecithin modified with hydrogen peroxide Weighted mean percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods, baking mixes</td>
<td>0.12</td>
<td>0.35</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>0.16</td>
<td>--</td>
</tr>
<tr>
<td>Grain products such as pastas or rice dishes</td>
<td>0.32</td>
<td>--</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>0.19</td>
<td>--</td>
</tr>
<tr>
<td>Milk, milk products</td>
<td>1.33</td>
<td>0.06</td>
</tr>
<tr>
<td>Cheese</td>
<td>0.38</td>
<td>--</td>
</tr>
<tr>
<td>Frozen dairy desserts, mixes</td>
<td>0.53</td>
<td>--</td>
</tr>
<tr>
<td>Processed fruits, juices, and drinks</td>
<td>0.01</td>
<td>--</td>
</tr>
<tr>
<td>Meat products</td>
<td>0.04</td>
<td>--</td>
</tr>
<tr>
<td>Poultry products</td>
<td>0.04</td>
<td>--</td>
</tr>
<tr>
<td>Fish products</td>
<td>0.03</td>
<td>--</td>
</tr>
<tr>
<td>Processed vegetables, juices</td>
<td>0.01</td>
<td>--</td>
</tr>
<tr>
<td>Soft candy</td>
<td>0.18</td>
<td>0.06</td>
</tr>
<tr>
<td>Sugar, confections</td>
<td>0.34</td>
<td>0.20</td>
</tr>
<tr>
<td>Sweet sauces, toppings, syrups</td>
<td>0.25</td>
<td>--</td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td>0.02</td>
<td>--</td>
</tr>
<tr>
<td>Soups, soup mixes</td>
<td>0.02</td>
<td>--</td>
</tr>
<tr>
<td>Snack foods</td>
<td>0.02</td>
<td>--</td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>0.83</td>
<td>--</td>
</tr>
<tr>
<td>Nuts, nut products</td>
<td>0.50</td>
<td>--</td>
</tr>
<tr>
<td>Reconstituted vegetable proteins</td>
<td>0.52</td>
<td>--</td>
</tr>
<tr>
<td>Gravies, sauces</td>
<td>0.18</td>
<td>--</td>
</tr>
<tr>
<td>Dairy products analogs</td>
<td>1.17</td>
<td>0.39</td>
</tr>
<tr>
<td>Hard candy</td>
<td>0.06</td>
<td>--</td>
</tr>
<tr>
<td>Seasonings and flavorings</td>
<td>0.01</td>
<td>--</td>
</tr>
<tr>
<td>Baby food formulas</td>
<td>0.08</td>
<td>--</td>
</tr>
</tbody>
</table>
and tocopherol may be present in trace amounts depending upon the refining process used. Oil-free lecithin has most of the triglycerides and free fatty acids removed and contains 90% or more of phosphatides. Edible diluents such as cocoa butter and vegetable oils often replace soybean oil to "improve functional and flavor characteristics". Food grade lecithin must contain not less than 50% acetone-insoluble matter (phosphatides), no more than 1.5% water, 0.3% benzene-insoluble material, 3 ppm arsenic, 40 ppm heavy metals (as lead), or 10 ppm lead. Its acid value must not exceed 36. The Food Chemicals Codex (National Research Council, 1981b) provides no specifications for bleached lecithins. The distribution of individual phospholipids in oil-free commercial soybean lecithin is shown for a typical preparation in Table A-1 (Erdahl et al., 1973). All in all there may be 17-20 classes of lipids in commercial oil-free lecithin.

The participants in the ad hoc review group concluded that the specifications for lecithin should be expanded (see pages 59-61).

C. PROPERTIES OF PHOSPHATIDYLCHOLINE

Phosphatidylcholine in purity greater than 95% is commercially available only in small quantities for research purposes. As noted on page 2, phosphatidylcholine is a term for a class of compounds which vary in the nature of the diacylglycerol residue. Specifications for a manufactured product should include:

1. source, if prepared from natural products or synthetics;
2. optical purity, giving percentage of natural enantiomer and its unnatural form;
3. distribution of fatty acids according to position of substitution;
4. nature and extent of impurities such as heavy metals and peroxide content.