

Nano-Bio-Technology and Bioavailable Phytonutrition

*“Knowledge of the role of physiologically active food components, from both **phytochemicals and zoochemicals**, has changed the role of diet in health. Functional foods have evolved as food and nutrition science has advanced beyond the treatment of deficiency syndromes to reduction of disease risk... Foods can no longer be evaluated only in terms of macronutrient and micronutrient content alone. Analyzing the content of other physiologically active components and evaluating their role in health promotion will be necessary. The availability of health-promoting functional foods in the US diet has the potential to help ensure a healthier population... fruits and vegetables represent the simplest form of a functional food...”* -American Dietetic Association on Functional Foods

The Poor Bioavailability of Phytonutrients

Fruits and vegetables are indeed rich in phytonutrients. However, the bioavailability of phytochemicals can be a challenge to those designing high quality functional foods and phytonutrient supplements.

“The low solubility of free ellagic acid (a phytonutrient of the organic acid class derived from fruits and nuts, especially raspberries and wild tart cherries) is thought to be due to its low solubility in water.” 1

“the bioavailability of quercetin-3-rutinoside is 17%” 2

“Bioavailability differs greatly from one polyphenol to another” 3

“In general,... epicatechins (polyphenols from green tea)... had low bioavailability...” 4

“Cyclic terpenoids-saponins and phytosterols have structures similar to ...steroids...(which are) more soluble in oil than water.” 5

“Delivery of natural carotenoids can be compromised by poor bioavailability.” 6

“Consuming tomatoes with oil can increase the bioavailability of lycopene” 7

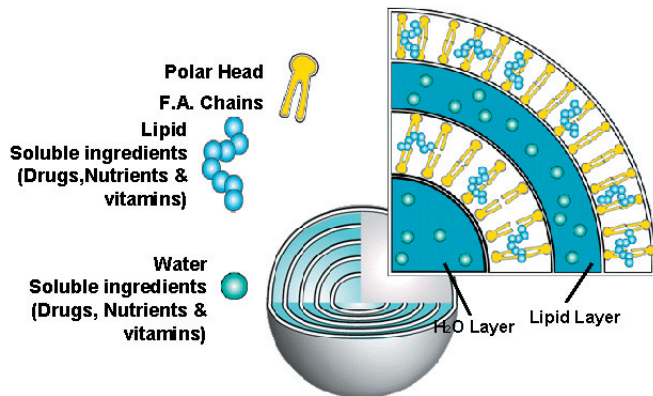
“Essentially no absorption of carotenoids was observed when salads with fat-free salad dressing were consumed.” 8

The above statements demonstrate a significant barrier to optimal benefits accrued in the use of phytonutrient supplements, “green drinks” and related supplemental phytonutrient drink mixes and powders. Nonetheless we do know that the human body is designed to absorb both water soluble nutrients and water insoluble fats, albeit in different ways. One strategy for enhancing the bioavailability of phytonutrients poorly soluble in water is to mimic the body’s own strategy in absorbing water insoluble fats and oils. This may be achieved biologically by the infusion of nutraceuticals and nutrients into nano-sized liposomes. (One nanometer is 1/1000 of a micron).

We will herein briefly review the structure of liposomes and the physiology of fat absorption so that the strategy of encapsulating phytonutrients in lipids nano-vesicles, or liposomes, to maximize bioavailability might be better appreciated.

What are Liposomes?

Liposome Architecture



Liposomes are self-assembled, self-closed colloidal nano-particles with membranes composed of a lipid bi-layer which encapsulate or infuse a percentage of the solvent in which they are suspended. Because liposomes can entrap hydrophilic molecules into their interior, and hydrophobic molecules into their lipophilic membrane, they have been used as a delivery system for pharmaceuticals, cosmeceuticals, vitamins and nutraceuticals.

The basic component of all clinically useful liposomes has been the phospholipid molecule. Phospholipids are amphiphilic molecules composed of a polar hydrophilic head group and two hydrophobic fatty acid chains attached to a three-carbon glycerol backbone.

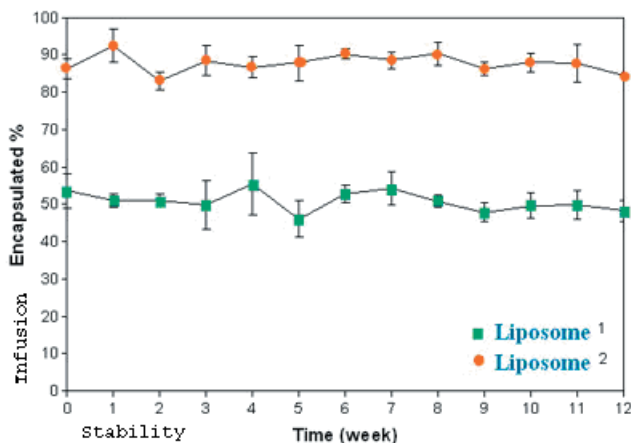
When phospholipids are mixed with water they form bi-layer sheets that can be turned into vesicles by adding ‘process energy’ in the form of high pressure mixing, or sonication. Liposomes may be described, therefore, as bi-layer nanovesicles separated by aqueous compartments. Liposomes are either unilamellar or multilamellar.

Stability of the liposomes depends upon the nature of the constituent lipid molecules used to construct them, as well as the composition and concentration of the inclusion material.



Typical Size Ranges: SLV: 20-50 nm – MVL:100-1000 nm

Various pharmacological agents of varying solubility and size have already been encapsulated in either the aqueous or the lipid phase of the liposomes. That percentage of encapsulation is called the “inclusion rate”. A high percent of inclusion (greater than 70%) of the target composition within the liposome is critical. Liposomal preparations having *high stability* and a *high inclusion* percentage significantly affect efficacy and cost.



The following chart compares stability and infusion rates. The latter is the percentage of successful liposomal encapsulation, for both older and newer encapsulation technologies, type I and type 2 respectively.

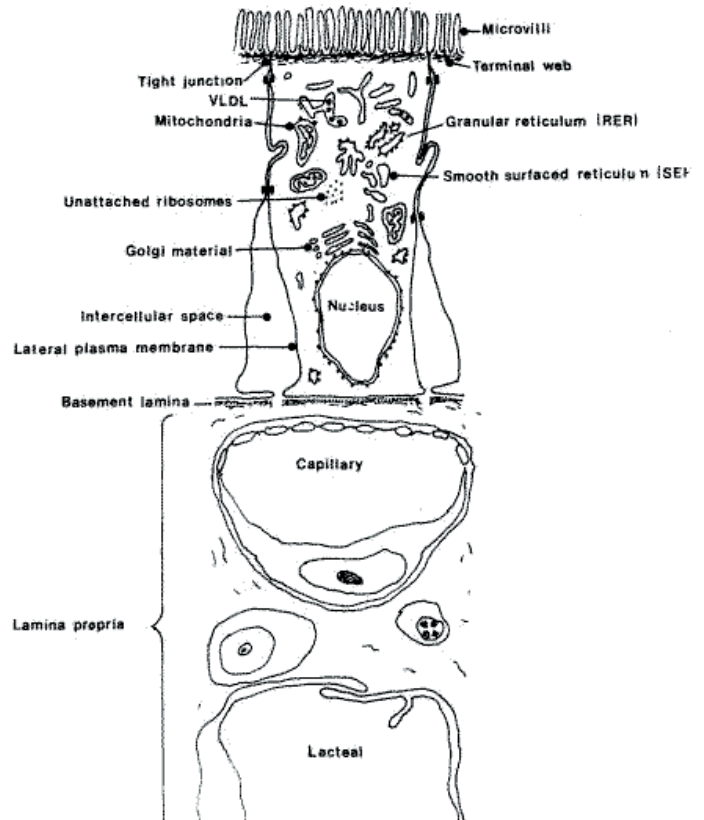
Comparative infusion & stability profile of CoQ-10 for 3 months at room temperature

Liposomal Functions in Digestive Physiology

Liposomes of less than 500 nanometers in size are rapidly absorbed through the intestinal villus. Nutrients encapsulated in liposomes are not only absorbed rapidly but utilize a different absorption strategy than standard water soluble route, or the method of a “nutrient assist” for either carbohydrates, proteins, or amino acids. A brief review of digestive physiology is therefore in order. 9

The small intestinal wall is made up of four layers: the inner mucosal layer; the submucosa of vessels and nerves, smooth muscle layer, and the outer serosal covering.

The main function of the mucosal enterocyte covered villi is absorption. The connective tissue of the mucosal and submucosal structures beneath the absorptive epithelium support it. They must be traversed before an absorbed nutrient can be removed via the portal venules or lymphatic lacteals. The figure below shows a cross section of the mucosal and submucosal layers.



Water soluble absorption

Intestinal cell membranes have lipoidal properties. Non-lipid molecules such as monosaccharides, amino acids, di- and tripeptides and electrolytes are polar and water soluble; they can be absorbed in only two ways: either through the lipid membrane assisted by carrier proteins (transcellular transport), or through gaps in the intercellular (“tight”) junctions (para-cellular junction).

Water insoluble absorption

Dietary fat is mostly long-chain triglyceride which is extremely insoluble in water (nonpolar). The human body is composed mostly of water, but its cell barriers are mostly lipid. Therefore, fat absorption is essentially a transport problem involving movement from water compartments through lipid membranes into water again. Specifically, the problem is how to dissolve or disperse fat widely in the water of the intestinal lumen and then facilitate its efficient transport to and through the absorptive cell’s lipid membrane. Once it is inside the cell, it has to be packaged in a multi-molecular form as chylomicrons which can be carried safely in water, lymph and blood as a finely dispersed stable emulsion.

Fat Digestion and Transport

To achieve this dispersion of fat in water, we start with gastric contractions to emulsify fats with the aid of food proteins and lecithins. As the chyme enters the duodeno-jejunum, it stimulates release of CCK and secretin from the duodenal mucosa and assures a continuous flow of bile and pancreatic juice for optimal luminal emulsification and digestion.

With the aid of the detergent action of bile salts, a whole spectrum of sizes of multi-molecular particles are formed. These are 200 nanometer or smaller diameter micelles and 500 nanometer or smaller diameter liposome aggregates composed of fatty acids, lecithins and monoglycerides.

The molecules of the lipid aggregates are so oriented that their polar groups face the surrounding watery medium which enables them to be widely dispersed or “dissolved” within the luminal contents. Their nonpolar interiors can dissolve lipids such as cholesterol and the fat-soluble vitamins (D, A, K and E and phytochemicals). Thus these lipid aggregates (micelles and liposomes) are vehicles to transport water-insoluble molecules through the watery luminal contents to the microvillous membrane of the absorptive cell through which the lipid nutrient molecules are absorbed.

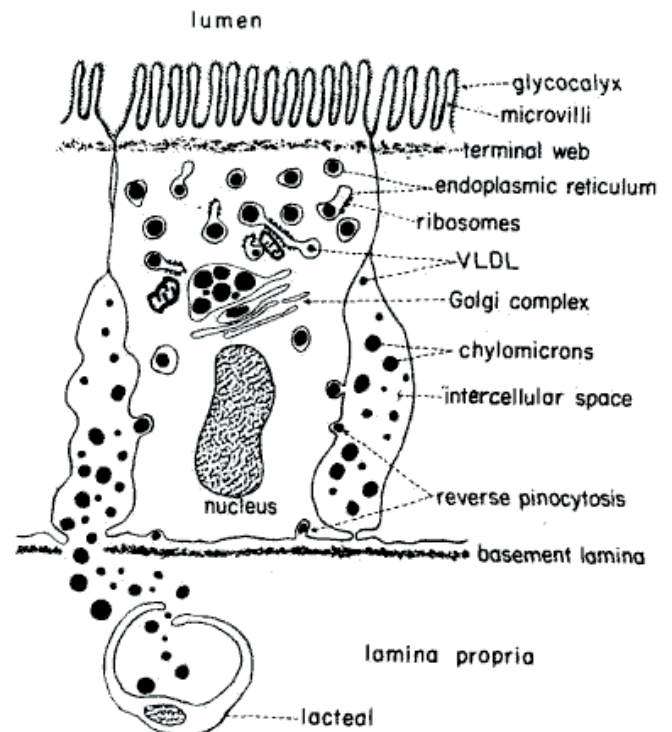
Furthermore, active absorption of liposomes, not micelles, seems to take place via the immune system.

“Liposomes (phospholipid artificial membrane vesicles)... it has been suggested..., when given orally, are taken up by M cells for delivery... to underlying lymphoid cells of the Peyer’s patch. This study investigated in vivo the uptake of liposomes by cells of Peyer’s patch... Sections of Peyer’s Patch from experimental animals showed M cells with endocytic

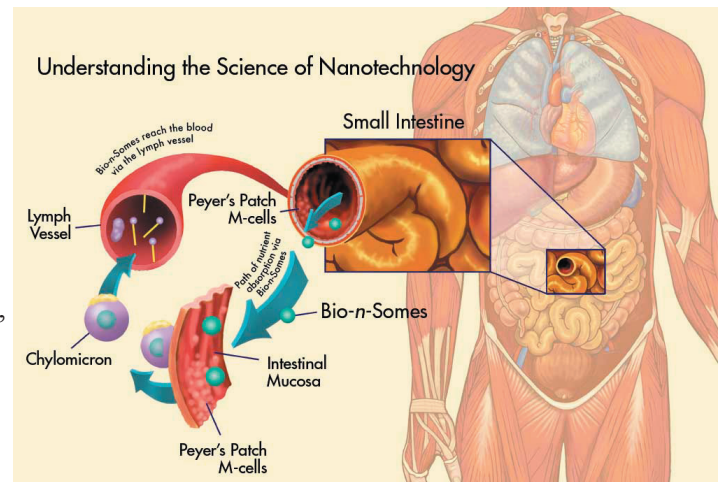
vesicles containing liposomes. Vesicles containing liposomes were also observed between M cells and lymphoid cells. These results indicate that intact liposomes are endocytosed by M cells...” 10

“...there is specialized tissue in the lower GI called Peyer’s Patches that contain M cells that sample for vesicles, not micelles or mixed micelles, but vesicles, like liposomes and chylomicrons. These cells will ‘pull’ in vesicles and their contents and dump them directly into the blood stream.” 11

Once absorbed by the mucosal layer of the small intestine, soluble nutrients easily reach the lumen of the capillaries in the submucosa. Fat soluble nutrients are first attached to chylomicrons and VLDL, which leave the absorptive mucosal cells, probably by reverse pinocytosis, and travel through the extracellular portion of the mucosal layers to enter the lacteals in the submucosa.



Solutes in the capillaries reach the liver via the portal vein. Lipid particles in the lacteals reach the systemic circulation via the thoracic duct which empties into the left subclavian vein. The nutrients carried by the chylomicrons are made available by the action of lipoprotein lipase. The enzyme lipoprotein lipase (LPL), bound to the surface of capillary endothelium, most especially in muscle and adipose tissue, frees the transported nutrients by hydrolysis. 12



New Nano-Bio-Technologies to Optimize Bioavailability

In conclusion, nanoencapsulating poorly bioavailable nutraceuticals in lipid liposomes mimics the body's own solution to absorbing poorly soluble nutrients. The challenge has been to more economically create liposomes that not only form spontaneously, but *encapsulate spontaneously* just by adding water at ambient temperatures, without solvents, pressure, sonication or shearing forces. The goals are unadulterated potency and purity of the nutrient/nutraceutical, with high inclusion rates, excellent stability, and proven bioavailability for water and fat soluble molecules, for both liquids and powders. Fortunately, in just the last decade breakthrough nano-technologies with proven efficacy and extremely favorable economy have been patented which outperform earlier liposomal technologies. High quality, high quantity liposomal delivery systems are now economically feasible for use in dietary supplements, medicines and functional foods.

Interestingly, this liposomal encapsulation strategy is applicable to sublingual and dermal applications as well which will deserve further elucidation in a subsequent article.

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