CHAPTER I

INTRODUCTION

It is well recognized that physical properties and functions of cellular membranes are influenced by their lipid composition (Dahlan, 1989). Polyunsaturated fatty acids (PUFA), which are the major components of the hydrophobic core of the membranes bilayer and found mainly esterified in phosphoglycerides, can interact with and directly modulate the functions of some membrane proteins involved in cellular communications and homeostatic processes, such as enzymes, receptors, and ion channels (Bayon et al, 1997). Besides of the above-mentioned functions as well as acting as the precursors of eicosanoids, PUFA's also affect membranes' shape and their ability to pack together. It is known that even very slight changes in phosphoglycerides fatty acids especially PUFA can cause marked changes in membrane properties. For example, the substitution of arachidonic acid or AA (C 20:4 n-6) by eicosapentaenoic acid or EPA (C 20:5 n-3) has marked effects on eicosanoid production in the membranes. This is especially true for the PUFA replacement in membranes of platelets which subsequently affect the hemostasis of the blood (Bayon et al., 1997; BNFTF, 1994) (see Appendix).

Platelet is one of blood cells that participate in hemostasis by forming aggregates at the site of vascular injury and by providing a source of phospholipoprotein to promote clotting. PUFA of platelet phospholipids are active in

establishing eicosonoids (Broekman et al., 1976) (see Appendix). Thus, PUFA on platelet membrane are necessary to affect platelet thrombogenesis, a major cause of cardiovascular disease (CVD) which is well established as one of the most important health problems in almost developed countries and some developing countries like Thailand. (Dyerberg et al., 1978; McPherson and Spiller, 1996).

In the past the curation and prevention of CVD was emphasized on lowering hypercholesterolemia of the patients. Today we know more about lipid metabolism and etiology of CVD. It has been proven that n-6 PUFA's provide disadvantageous effect for the prevention of CVD in some circumstances, i.e. lower HDL, produce proatherogenic and prothrombogenic eicosanoids (Grimminger et al, 1995) (see Appendix). Now the important functions of n-3 PUFA's, particularly EPA and DHA from fish or fish oil, in atherogenesis, inflammation, thrombus formation, gene expression, and cell-to-cell communication have taken central stage and have led to intervention studies and clinical trials in CVD and many diseases (Connor, 1997). Hence, the results accumulated from several epidemiological studies are likely to point out that in order to prevent population from CVD, the PUFA composition of platelet membranes with high n-3 to n-6 PUFA ratio is thus preferential (Dahlan et al., 1997; Bayon et al., 1997).

While the composition of membrane lipids is less susceptible to dietary fatty acids than that of storage lipids, modification of the fatty acid composition by dietary fatty acids is well established and, in recent years, has been particularly well studies for PUFA of the n-3 and n-6 series. However, modifications of the cell phospholipid fatty acid composition induced *in vivo* by dietary means is time-

consuming. Furthermore, the dietary means still affects so many lipid molecular species that it is difficult to correlate these changes with cellular functions and membrane physical properties (BNFTF, 1994). Alternatively, the lipid composition of liposomes or fat emulsion particles can induce much more rapid alteration of membrane fatty acid composition. Dahlan et al. (1992b) demonstrated *in vivo* that daily intravenous infusion for three months of fat emulsions rich in n-6 PUFA in both emulsion's core and surface induce the decrease of n-3 to n-6 fatty acid ratio in erythrocyte membranes of the patients with inflammatory bowel disease. Dahlan also demonstrated *in vitro* that the n-3 to n-6 fatty acid ratio of erythrocyte membrane could be altered within 1 h of incubation to the preferred value when the novel emulsion rich in fish meal-derived lecithin was utilized as n-3 PUFA donor (Dahlan et al., 1997).

In the present experiment, n-3 PUFA rich lecithin was again produced as lecithin-rich fat emulsion and utilized as n-3 PUFA supplier. Differently from our previous experiment, platelets with much more delicate techniques of preparation and handling than those of erythrocytes were used as n-3 PUFA recipient. The exchanges of surface fatty acid between platelets and fat emulsion with triacylglycerols (TG) core rich in n-3 PUFA was also studied in order to investigate the effect of TG core on the surface PUFA interaction.

Aims of the Present Study

In our present study, we aimed to augment n-3 to n-6 PUFA ratio of platelets *in vitro* by utilizing fat emulsions with n-3 PUFA rich lecithin derived from fish meal as

emulsifier. The subsequent altered function of platelet membranes especially eicosanoid production was speculated without any investigation included in the study.

Hence, the objectives of the present study are:

1. to prepare fish meal lecithin with high n-3 PUFA contents and to study its lipid characteristics.
2. to prepare a fat emulsion rich in n-3 PUFA by utilizing fish meal lecithin.
3. to establish the condition for the incubation of platelets with fat emulsions: effect of plasma on PUFA transfer and the stability of transferred fatty acids.
4. to study the effect of the prepared fat emulsions on the exchanges of their fatty acids with platelets by comparing the results with fat emulsion derived from soya and egg yolk.
5. to prepare fat emulsion of soya lecithin coring inside with fish oil and study the effect of fish oil in the core on fatty acid transfers on the surface.