Introduction
Myocardial ischaemia occurs when there is a severe reduction of coronary flow i.e. the supply of oxygen to the myocardium is inadequate for the oxygen demands of the tissue. An emerging biological concept proposes two phases of adaptations for the myocardium to recover from severe ischaemia. They are (1) short term defense and (2) long term rescue (Hochache et al., 1996).

The aim of short term defense is to achieve a new balance between the oxygen demand and supply by a combination of down regulation of contraction and upregulation of anaerobic energy production by glycolysis. Long term rescue is still poorly understood but increasingly it seems that ischaemia perhaps acting through hypoxia is able to induce a series of cellular signals that lead to protective genetic reprogramming (Knoll et al., 1994). When defence and rescue fail because the ischaemia is overwhelmingly severe and prolonged, then the result is cell necrosis.

Acute myocardial infarction is caused by an imbalance between myocardial oxygen supply and demand of sufficient duration that myocardial necrosis ensues. Often it is the consequence of a dynamic process within the coronary vessel initiated by the thrombotic occlusion of a previously stenotic coronary artery (Dewood et al., 1980).

The term "infarction" literally means "stuffed in" and refers to the swollen appearance of totally dead cells. The time taken for the whole sequence to develop is only 20 to 60 minutes when there is little or no collateral flow and myocardial oxygen uptake is relatively high. In contrast, the time is
Fig. 1.1 Acute myocardial infarction is a clinical syndrome in which a significant number of ischemic cardiac cells eventually die. Although in the early stages the condition is reversible and constitutes ischemia and not necrosis, clinicians include such reversible early ischemia in the overall syndrome of AMI. If the thrombus is broken down by therapeutic thrombolysis, the threatened myocardium can be salvaged. The therapeutic window is that time between the onset of chest pain and irreversible ischemia. In the presence of collateral flow and a low myocardial oxygen uptake (MVO₂), animal data suggest an interval of 2 to 6 hours, whereas in the presence of no collateral flow and a high oxygen uptake, the interval is 20 to 60 minutes.
extended to 2 to 6 hrs when the collateral flow is high and the myocardial oxygen uptake is low (Schaper et al., 1987).

The infarcts can be categorised as regional transmural (RTM), regional subendocardial (RSE) and diffuse subendocardial (DSE).

Pathological studies of regional transmural infarctions have revealed that the supplying artery is totally occluded in over 90% of cases, suggesting that persistent occlusion is related to greater risk of fatal outcome possibly because of larger infarct size (Kim et al., 1993).

At the proximal end of the occlusion within the intima there is a mass of predominantly platelet thrombus which is contiguous through a fissure in the cap with the main mass of thrombus within the lumen. Adjacent to the plaque fissure the thrombus has a high platelet content but more distally there is a higher fibrin and red blood cell content. The microstructure of the thrombus is consistent with a phasic progression and the distal propagation is of a 'stasis' type, suggesting that thrombus growth continued after the vessel was occluded.

In the subendocardial infarction there is a recent plaque disruption in the supplying artery but there is far lower incidence of total arterial occlusion (DeWood et al., 1981). There is a high incidence of the distal segment of the artery being patent and filling either by antegrade flow over mural thrombus or more rarely by well developed collateral flow. Distal propagation of thrombus is virtually never found in non transmural infarction.
Diffuse (non-regional) subendocardial necrosis may occur in man in the absence of any structural coronary artery obstruction and reflects an overall fall in myocardial perfusion accentuated in its effect on the subendocardial zone. Subendocardial muscle, as compared with subpericardial muscle, has a 20% higher oxygen utilization per gram of tissue, produces lactate readily, undergoes necrosis first under ischaemic conditions and, in conscious animals, has a higher resting blood flow (Hoffman, 1987).

The physiological basis of subendocardial underperfusion has been shown to result from the higher pressure generated in the subendocardial zone in systole; as a result, the intramyocardial vessels of this zone are empty and offer greater impedance to reflow in diastole. Diffuse subendocardial necrosis may develop when regional infarction is complicated by cardiogenic shock. The central zones of papillary muscle are the most vulnerable component of the subendocardial tissues and undergo necrosis first.

**Risk factors for cardiovascular disease (CVD)**

The various cardiovascular risk factors include:

**Elevated blood pressure:** Blood pressure is an important risk factor for cardiovascular disease and reduction of blood pressure levels results in a significant decrease in cardiovascular disease.

**Lipids and lipoproteins:** Dyslipidemia has been shown to be associated with increased risk of Coronary heart disease (CHD) in many
studies (Shekelle et al., 1981; Reed et al., 1986; Castelli et al., 1984; Rose et al., 1986). The lipid research primary prevention trial data documents the importance of blood lipids as a risk factor for cardiovascular disease and that improvement of lipid profile results in an improvement in risk of CVD (Lipid Research Clinics Programme, 1984).

**Cigarette smoking and cardiovascular disease:** Cigarette smokers are at increased risk of both myocardial infarction (MI) and sudden death. The likely mechanism for the association between cigarette smoking and CHD is related to the increased thrombogenesis and decreased oxygen carrying capacity in cigarette smokers. Longitudinal studies have shown that the risk of MI decreases after smoking cessation and that the improvement in risk occurs rapidly after cessation of cigarette smoking (Salonen, 1980).

**Diabetes and cardiovascular disease:** Patients with diabetes mellitus are at increased risk of CVD. The increased risk in diabetes appears to be mediated in part by the increased prevalence of hypertension and dyslipidemia. Recent data suggest that increased insulin resistance and hyperinsulinemia in diabetics may be associated both with their abnormal CVD risk profile and with their increased risk of CHD (Reaven, 1991; Manolio et al., 1991).

**Coagulation factors in cardiovascular disease:** Alterations in hemostatic factors may mediate in part the relationship between smoking and cardiovascular morbidity and mortality, emphasizing the importance of chronic factors associated with atherosclerosis and acute factors associated with risk of a thromboembolic episode precipitating the acute CHD event. Levels of
fibrinogen and factor VII activity have been associated with increased risk of CHD events (Meade et al., 1986; Kannel et al., 1987).

**Left ventricular hypertrophy (LVH) and cardiovascular disease:** LVH has been linked to increased risk of CVD. Framingham data have suggested that LVH not only is independently related to CVD, but also that it is one of the strongest CVD risk factors in their study (Levy et al., 1990).

**Obesity and cardiovascular disease:** Evidence exists documenting the presence of an independent relationship between obesity and CHD (Hulbert et al., 1983). In addition obesity has been associated with a more adverse CVD risk profile. Obesity has been linked to a greater prevalence of hypertension, dislipidemia and abnormalities in glycemic status (Smoak et al., 1987; Najjar et al., 1987; Gillum et al., 1987). A modest weight loss results in significant decline in risk factor (Fletcher, 1954). Thus, obesity is both an independent CHD risk factor and a marker for higher levels of established CHD risk factors.

**Physical activity and coronary heart disease:** The observed inverse relationship between physical activity and CHD likely manifests itself through known cardiovascular disease risk factors. Cohort studies have shown that persons who are more physically active have a lower risk of subsequent CHD than do those who are not physically active (Pamrehn et al., 1982). At least part of the direct association between exercise and CHD is mediated by a lower prevalence of cigarette smoking, lower levels of blood pressure and an improved lipid profile.
Fig. 1.2 Structure of Isoproterenol
Diet and Coronary Heart Disease: Numerous studies have shown an ecologic relationship between dietary intake and prevalence of CHD in different countries. Populations that consume a high percentage of their calories from fat are at increased risk of heart disease. Likewise, increased consumption of dietary sodium has been linked to an increased prevalence of hypertension in studies across populations (Stamler et al., 1989). Thus dietary factors play a key role in the genesis of CVD risk factors and, hence, risk of CVD.

Experimental Induction of Myocardial Infarction

Animal models of AMI have generally used either acute occlusion by ligature of otherwise healthy coronary arteries (Hock et al., 1987) or massive injections of catecholamines (Wexler, 1973).

Administration of isoproterenol [1-(3,4 dihydroxy phenyl 2 isopropyl amino ethanol)] a synthetic catecholamine and a β adrenergic agonist has been reported to produce gross and microscopic infarct like damage in rats when administered in large doses (Rona et al., 1959). There was a close correlation between the dose injected and the degree of severity of necrosis thus allowing production of standardised myocardial lesions. The lesions produced by isoproterenol were most severe, consisting of infarct areas of myocardium and necrosis with hyalinization or vacuolization of the muscle fibres, interstitial and cellular oedema and extensive inflammation (Ferrans et al., 1964).
The pathophysiological changes in isoproterenol induced myocardial infarction are comparable to that of myocardial infarction in humans. Distress and tachypnoea following isoproterenol administration are similar to the clinical features seen in human myocardial infarction. Wexler and Kittinger have also reported similar findings in rats following subcutaneous administration of isoproterenol (Wexler et al., 1963). The drug has positive chronotropic and inotropic actions because isoproterenol activates β-receptors almost exclusively and is a potent vasodilator.

Isoproterenol being a β-adrenergic agonist, increases the heart rate, force of myocardial contraction, atrial and ventricular conduction. It decreases peripheral resistance and increases cardiac output. The increase in heart rate usually accompanies an increase in myocardial oxygen consumption. Tachycardia which ensues after isoproterenol administration not only increases oxygen demand but also decreases myocardial oxygen supply because of diastolic filling time of the coronary circulation. Thus it lowers the end diastolic coronary perfusion pressure. The intensity of tachycardia outstrips the ability of coronary arteries to provide adequate blood supply to the myocardium. Thus a stage of relative myocardial ischaemia develops which results into necrosis (Opie, 1975; Maroko et al., 1976).

Isoproterenol induced cardiac necrosis, include increased myocardial contractility, increased oxygen consumption, alterations of membrane permeability, increased calcium overload and accumulation, increased intracellular acidity and free fatty acids, increased myocardial cAMP
**β-adrenergic messenger system** (Wollenberger A, 1975). Between the first messenger and the end result is a complex sequence of events. Thus, catecholamine stimulation of adenylyl cyclase eventually enhances calcium uptake by the sarcoplasmic reticulum (SR). Similar principles apply to other end results. R, regulatory; C, catalytic subunits of protein kinase A.
concentration, changes in intermediate cardiac cell metabolism, deranged electrolytes milieu and mechanical or dynamic hinderance of coronary circulation.

The mechanism of isoproterenol induced myocardial lesion is probably by myocardial calcium overload and consequent high energy phosphate depletion. The high energy phosphate depletion is directly proportional to the degree of myocardial damage induced by isoproterenol (Fleckenstein, 1983).

The basic molecular events in isoproterenol induced myocardial lesion have been described by Wollenberger. Catecholamines through the formation of cAMP activate a membrane bound protein kinase, which in turn induce the phosphorylation of sarcolemmal membrane proteins. By this process, the number of fixed negative charges that can recruit Ca\(^{2+}\) uptake from the extracellular space is increased. Isoproterenol augments the capacity of the superficial Ca\(^{2+}\) store so much so that the Ca\(^{2+}\) uptake from the environment and the subsequent transsarcolemmal influx are greatly enhanced. This process results in cellular Ca\(^{2+}\) overload and ultimately leads to myocardial necrosis (Wollenberger, 1975).

**Electrocardiographic changes in AMI**

As ischaemia is prolonged and irreversibility develops, so does a new series of electrocardiographic (ECG) changes characterized by Q waves and an acute infarction pattern. The very early ECG changes of hyperacute infarction during the stage of myocardial ischaemia are those of ST segment deviation,
which is a positive deflection in the case of epicardial damage and a negative deflection in the case of endocardial damage. The ST changes are those of depolarization caused by ischaemic potassium ion shifts. Next, as the tissue undergoes necrosis, the electrode "sees" through the dead myocardium to look at the other wall of the ventricle, where the major electrical force moves in the opposite direction from the endocardium through the ventricular wall. Thus, the exploring electrode sees a negative deflection, that is a Q wave. In this process, the R wave will first fall and decrease in magnitude before the Q wave becomes evident.

The extent of the change of the typical ECG pattern from early ST elevation to Q wave formation 4 to 12 hrs after the onset of ischaemia provides an indirect index of myocardial necrosis. Theoretically, the efficacy of an intervention reducing experimental infarct size can be tested in humans by its capacity to lessen the development of ECG signs of necrosis, such as loss of frontal forces (decrease of R waves) and formation of Q waves, which occur over about 4 to 12 hrs.

Clinical diagnosis of AMI

It is often important to distinguish patients with true AMI from those who have other sources of chest pain and among those with true AMI to diagnose irreversible damage. It is important to know whether thrombolytic reperfusion has taken place. The release of intracellular enzymes and contractile proteins into the circulation is helpful in these endeavours.
The diagnostic makers for acute myocardial infarction are creatine kinase, lactate dehydrogenase, aspartate amino transferase and alanine amino transferase. Each enzyme shows a different time course for release into the plasma and subsequent disappearance, these differences depend on the concentration of each present in cardiac tissue, their subsequent rate of release and clearance (Horder et al., 1979).

Creatine kinase is the earliest enzyme to be detectable rising 4-6 hrs after the onset of acute myocardial infarction reaching peak between 24-36 hours and then rapidly declining, alanine amino transferase reaches a peak between 48 hrs and 60 hrs, the LDH between 48 and 72 hrs. The prognosis and progress can be assessed by the increase in the activities of these enzymes. Creatine kinase can be particularly useful in the early detection and prognosis of myocardial infarction.

Creatine kinase of cardiac origin (CK-MB) is specific to the heart and can be detected within hours of the onset of AMI, but increased sensitivity and specificity is obtained by the assay of subforms of CK-MB. There is only one form of CK-MB in the heart, namely CK-MB₂, but this is converted in the blood to CK-MB, which can be detected within 6 hrs of the onset of symptoms (Puleo et al., 1994).

Increases in blood levels of myoglobin and troponin T are both candidates for the early diagnosis of AMI. For the purposes of ruling out AMI (often very important for cardiologists) early release of myoglobin is better (De Winter et al., 1995).
Fig. 1.4 Mechanism of infarction. Proposed metabolic mechanisms whereby ischemia can produce infarction. The two major effects of ischemia are poor O₂ delivery (hypoxia) and poor washout of metabolites. Depressed mitochondrial metabolism results in decreased production of ATP and accumulation of fatty acid metabolites, which are normally metabolized in the mitochondria. Anaerobic metabolism causes accumulation of lactate and protons (the later from breakdown of ATP), and continued residual respiration causes accumulation of CO₂. Decreased production of glycolytic ATP (a result of accumulation of lactate and/or protons) results in calcium accumulation. Inhibition of ion pumps by lack of ATP and by inhibition of fatty acid metabolites results in potassium loss, as well as sodium and water retention with cell swelling (shown at right). Fatty acid metabolites also cause membrane damage, which may result also from lysosomal activation by severe cellular acidosis. The final events leading up to infarction may be increasing ischemia caused by ischemic contracture, mitochondrial damage, enzyme loss as a result of membrane damage, and proteolysis from lysosomal activation.
Mechanisms of irreversible ischaemic damage

The exact mechanism whereby reversible ischaemia finally evolves into irreversible infarction is still controversial. Both the consequences of poor oxygen delivery and poor washout metabolites may play a role, with the former probably being the predominant factor. Five current theories for the development of irreversible ischaemic damage are (1) loss of a critical amount of ATP, (2) membrane damage induced metabolically or mechanically, (3) formation of free radicals, (4) calcium overload, and (5) sodium pump inhibition.

A critical level of ATP can be excluded as the sole cause of irreversibility because of the different values for the critical level given by different investigators and because of the established concept of ATP compartmentation (mitochondrial versus cytoplasmic). It is impossible to say which compartment is depleted by measurements of overall ATP. However, the ATP theory is being revived and extended with the recent evidence that ATP produced by glycolysis may have specific role in the prevention of membrane, related ischaemic events, such as calcium influx (Owen et al., 1990) and potassium loss (Weiss et al., 1985). Inhibition of glycolysis markedly accelerates cell death in dogs with coronary occlusion (Sebbag et al., 1996). Thus, inhibition of glycolysis is postulated to be a crucial event in the progress to ischaemic cell necrosis.

Inhibition of sodium pump may precipitate an excess of internal sodium, which in turn leads to an increased osmotic pressure, which helps to "Pop" the cell membrane and to cause irreversible damage (Jennings et al., 1986). The
Fig. 1.5 Membrane damage in ischemia

- FFA: free fatty acids
- TG: triglyceride
- MITO: mitochondrial metabolism
- O_2-.OH: oxygen-derived free radicals
proposed cause of the pump failure is inadequate synthesis of sufficient glycolytic ATP for the pump (Cross et al., 1995).

Membrane damage is multifactorial in origin, and includes accumulation of free fatty acids inside and outside the ischaemic cells and increased amounts of acyl CoA and acyl carnitine. There may be a self perpetuating cycle whereby part of the membrane damage results from the action of phospholipases that break down membrane lipids, with formation of lysophoglycerides, which further promote membrane damage.

Oxygen free radicals also may contribute to membrane damage by lipid peroxide formation. Free radicals are derived in part from neutrophils, particularly during the reperfusion phase of ischaemic damage, and in part from damaged myocyte mitochondria.

The calcium overload concept of irreversibility has received special prominence in relation to conditions of massive calcium overload, such as catecholamine stimulation, severe reperfusion damage, or the very unusual experimental condition of the calcium paradox, whereby extracellular calcium is totally removed and then, when reintroduced, causes massive cellular damage. The basic concept of such severe degrees of calcium overload is that the mitochondria initially act as a buffer to take up calcium from the cytosol, which requires considerable energy. As a consequence, generalized cellular energy depletion is enhanced, the energy required for maintenance of ionic gradients becomes inadequate and membrane integrity is lost. A modified form of this hypothesis is as follows. In ischaemia, cytosolic calcium levels increase,
possibly as a result of intracellular redistribution of calcium. Such cytosolic calcium increases can activate phospholipases, increase resting tension and provoke fatal arrhythmias (Reimer et al., 1977).

Irreversibility (MI) probably depends on no single metabolic event, but like the gradual death of a person, it may be a complex phenomenon resulting from the simultaneous operation of many diverse mechanisms.

Interventions that reduce myocardial injury include:

Limitation of Infarct size

After abrupt coronary artery occlusion, irreversible cellular injury begins within the first 30 minutes in the subendocardium of the central ischaemic zone and spreads outward. The time for completion of the infarction varies, depending on the extent of collateral blood flow, haemodynamics and whether transient antegrade flow (opening and closing of the infarct artery) occurs. Because the ultimate extent of myocardial necrosis is the key factor determining short and long term prognosis after AMI, interventions to limit infarct size must be initiated as early as possible.

Thrombolytic agents

The most direct approach to the limitation of infarct size involves the restoration of coronary perfusion. Thrombolytic therapy with streptokinase, urokinase, tissue-type plasminogen activator or anisoylated plasminogen streptokinase activator complex has been demonstrated to restore arterial
patency in 80% of patients treated within the first few hours of AMI. Recent
data support the use of these agents up to 12 hours of after the onset of
infarction. Mortality is clearly related to both the extent and promptness of
recanalization (Eaton et al., 1979; Italian Group for the study of streptokinase
in myocardial infarction (GISSI), 1986).

**Antianginal agents**

Both β-adrenergic blockers and calcium antagonists act beneficially by
altering the supply-versus-demand equation. β-adrenergic blockers act by
decreasing the heart and contractile state of the myocardium, whereas calcium
antagonists act at least in part by relief of exercise-induced coronary
vasoconstriction (Frielingsdorf et al., 1996). Both types of agents also reduce
the afterload by decreasing the blood pressure.

**Nitric oxide**

Nitrates are thought to act in part as coronary dilators and in part as
vasodilators, thereby reducing the venous return to heart and lessening the
endocardial wall stress and its associated increase in oxygen demand. Because
nitrates are nitric oxide donors and the nitric oxide appears to inhibit
mitochondrial metabolism, which will decrease the oxygen demand
(Laursen et al., 1997). Agents indirectly producing nitric oxide, such as the
angiotensin-converting enzyme (ACE) inhibitors, which decrease the break
down of bradykinin and stimulate the endothelium to form nitric oxide, may
also reduce myocardial oxygen demand.
Clot specific treatment

This includes antiplatelet drugs, heparin and fibrinolytic therapy.

Antiplatelet drugs

Aspirin can reduce the incidence of MI and mortality in patients with unstable angina (Lewis et al., 1983; Cairns et al., 1985; Theroux et al., 1988). It protects against myocardial infarction by reducing platelet aggregation. Its mechanism of action seems to be inhibition of arachidonic acid metabolism. Ticlopidine is another antiplatelet agent which has shown similar benefit in patients with unstable angina (Balsano et al., 1990).

Prostacyclin (PGI2) a vasodilator is also being used clinically as an antiplatelet agent (Ribeiro et al., 1981).

Heparin

The usefulness of heparin has also been documented in the acute phase of unstable angina. Heparin and not aspirin can control severe recurrent ischemia (Neri Serneri et al., 1990).

Fibrinolytic therapy

The role of fibrinolytic therapy in unstable angina remains to be more accurately defined as to which patients will benefit from this therapy. Though it is found useful in successful treatment of some patients (Nicklas et al.,
1987). Overall a minority of patients benefit clinically from therapy (Lambert et al., 1989).

A possible explanation for the relative failure of fibrinolysis in unstable angina is a transient beneficial effect outlasted by an ongoing active disease process; also, clot bound thrombin, exposed by partial fibrinolysis, could lead to further platelet aggregation and thrombus formation.

**Magnesium**

A variety of clinical studies and experimental studies have suggested that intravenous administration of magnesium improves prognosis after AMI (Horner, 1992; Teokk et al., 1991).

**Metabolic mechanisms**

Although ischaemia is basically a metabolic problem, so far no metabolic therapies have been truly successful for effort angina, unless the metabolic effects of β-adrenergic blockade are considered. A possible exception is the metabolically active antianginal agent trimetazidine, which has no haemodynamic action but may act by lessening intracellular acidosis or by limiting sodium proton exchange.

**Other approaches to the limitation of infarct size include:** Hyaluronidase acts by breaking hyaluronic acid in the interstitial space so that substrate can diffuse more readily through the interstitial space towards ischaemic myocardial cells (Maroko et al., 1972).
Glucose Insulin Potassium (GIK): GIK was shown to reduce myocardial cell death by enhancing energy production in moderately ischaemic myocardium by stimulating anaerobic glycolysis, GIK infusions have been shown to alter myocardial substrate utilization from lipid to carbohydrate, to increase coronary sinus blood flow and decrease coronary vascular resistance in patients with coronary artery disease (Rogers et al., 1977).

Glucocorticoids: The rationale for using these drugs is that they stabilize lysosomal membranes, thereby inhibiting the release of lysosomal enzymes, which could play a role in cellular necrosis; It also stabilizes other cell membranes and decrease the inflammatory response which occurs during myocardial infarction (Shatney et al., 1976).

Oxygen free radical scavengers: These include various antioxidant vitamins A, B, C, β-carotene (Singh et al., 1996) and E. Potent agents at the early stages of clinical investigation include: specific inhibitor of platelet receptors, IIb - IIA and the specific thrombin inhibitors such as hirudin and related peptides.

Fish oil in coronary heart disease: Epidemiological studies indicate that death from cardiovascular disease is inversely related to the amount of marine food products consumed. This beneficial effect of sea food has been related to its high content of n-3 polyunsaturated fatty acids (Kromhuout et al., 1985).
Fig. 1.6 Graphic formula of the n-3 polyunsaturated fatty acids: cis-5,8,11,14,17-eicosapentaenoic acid (EPA; $C_{20}H_{30}O_2$) [top] and cis-4,7,10,13,16,19-docosahexaenoic (DHA; $C_{22}H_{32}O_2$) [bottom]
The n-3 fatty acids (also termed omega-3 fatty acids) are highly unsaturated fatty acids that are prominent constituents of marine oils. These fatty acids originate in plankton and extend up the food chain from krill to fish and then may be ingested by man. Biologically active n-3 fatty acids include eicosapentaenoic acid (EPA) (20:5 n-3) and docosahexaenoic acid (DHA) (22:6 n-3). These two fatty acids are present in relatively low amounts in the flesh of fatty fish but are concentrated in the various commercially available forms of fish oil (i.e. 20 to 60%) and may also be highly purified (eg upto 95%) as individual ethyl esters for research or therapeutic use (Scott et al., 1992).

The omega 3 fatty acids are important components of practically all cell membranes and they are essential for normal human growth and development (Hardy et al., 1994). EPA and DHA are essential fatty acids since they cannot be synthesized de novo in the body and are required for avoiding signs and symptoms of deficiency. Maintaining optimum proportion of these fatty acids in diet is essential.

Chemically EPA is cis-5, 8, 11, 14, 17-eicosapentaenoic acid ($C_{20}H_{30}O_2$, molecular weight 302.5) and DHA cis-4, 7, 10, 13, 16, 19-docosahexaenoic acid ($C_{22}H_{32}O_2$, molecular weight 328.5). Therapeutically, they have been categorized as hypolipidemic drugs, antiplatelet agents and cardioprotectants. They can be analysed in plasma or serum through chromatographic methods (Leibich et al., 1991).
Pharmacokinetics

Upon oral administration, EPA and DHA are absorbed from the intestine by the lymphatic route. The rate of absorption varies with the form in which they are administered. Although bioavailability may not differ, absorption may be faster in concentrated free fatty acid form than in triglyceride or alkyl ester form (Ikeda et al., 1993; Beckermann et al., 1990). The absorption may demonstrate dose dependence, particularly in case of EPA (Krokan et al., 1993; Marsen et al., 1992). When oils are administered the n-3 PUFAs are probably better absorbed from those containing triglycerides with a more symmetrical intramolecular structure (Christensen et al., 1995).

Following administration, EPA and DHA contents of serum increase in a dose-dependent fashion. Free and total EPA levels can rise by 5 to 6 fold or even higher. DHA levels however tend to peak at 2 to 2.5 fold baseline values. The rise is evident within a week of starting supplementation and the elevated levels may persist for several days or weeks following its discontinuation (Hansen et al., 1998). Supplementation also results in a rise in the EPA to arachidonic acid ratio and some decrease in the linoleic acid level in serum (Krokan et al., 1993).

Interconversion of unesterified polyunsaturated fatty acids, taken up from circulation, occurs in various tissues, including the liver and the gastrointestinal tract. There is interconversion between EPA and DHA. The polyunsaturated fatty acid, α-linolenate (18:3 n-3), is rapidly converted to EPA to substantial extent (Nilsson et al., 1995), indeed it is regarded as a precursor
Fig. 1.7 Schematic presentation of the involvement of phospholipase A2 in the production of eicosanoids from 20:4n-6 and 20:5n-3. Eicosanoids formed with 20:5n-3 as a precursor are given in parentheses. Depending on the precursor molecule, the eicosanoids will contain different numbers of double bonds and display different biological activity [7]. In general, the 20:5n-3 derived eicosanoids are weaker agonists in comparison to the analogous compounds derived from 20:4n-6.
fatty acid to EPA and DHA. Essential fatty acids can also undergo desaturation-chain elongation inside the cell. Supplementation with omega-3-fatty acids can decrease the formation of interconversion products from omega-6-fatty acids and vice versa (Hrelia et al., 1995).

Metabolism

A major metabolic fate of the n-3 PUFA is competition with arachidonic acid in cell membrane phospholipids for metabolism by the cyclooxygenase and lipoxygenase pathways (Lee et al., 1991). However, they do not inhibit the release or reacylation of arachidonic acid in activated cells (Lokesh et al., 1994). Incorporation of EPA into cell membrane phospholipids causes competitive inhibition of cyclooxygenase and decreases the conversion of arachidonic acid (20:4 n-6) to prostaglandins (Gryglewski et al., 1979). Similarly, eicosapentaenoic acid also inhibits the formation of leukotrienes by lipoxygenase (Lee et al., 1984; Lee et al., 1985; Prescott, 1984). Simultaneously, eicosapentaenoic acid acts as a substrate for these enzymes and is metabolized to triene prostaglandins and modified leukotrienes, which have significantly less biologic activity than their omega-6-derivatives (Fischer et al., 1985; Terano et al., 1988).

Cardioprotective effects of fish oil

**Myocardial protection:** Myocardial protection against ischaemia-reperfusion injury and related arrhythmias were well documented. Rather than acute treatment preventive benefit is more apparent here. Evidence has been
obtained from studies with cultured myocytes (Li et al., 1997), animal experiments (Yang et al., 1993), including those in non human primates (McLennan et al., 1993), clinical trials (Leaf et al., 1996) and epidemiological surveys (Siscovick et al., 1995; Albert et al., 1998). The ability of n-3 PUFA to prevent ischaemia induced ventricular fibrillation and sudden cardiac death is considered to be very important (Billman et al., 1994; Billman et al., 1997). In patients of coronary heart disease consumption of n-3 fatty acids lowered frequency of anginal attacks, reduced nitrate consumption and improved exercise tolerance (Salachas et al., 1994; Aucamp et al., 1993). Reduced infarct size has been found in those who sustained acute myocardial infarction (Landmark et al., 1998). A survival advantage of fish oil treatment has been documented in Indian patients with acute myocardial infarction (Singh et al., 1997).

**Antiatherogenic and antithrombotic role:** Epidemiological studies have long pointed to an antiatherogenic potential of n-3 fatty acids (Krombhoug et al., 1985; Kromhout et al., 1995). Animal experiments indicate that fish oil retards the growth of atherosclerotic plaque by modifying the local milieu of cellular growth factor and the behaviour of platelets and the vascular endothelium (Gusain et al., 1994).

Fish oils containing n-3 PUFAs have antithrombotic activity attributable to antagonism of platelet aggregation and possibly to profibrinolytic changes in the coagulation system (Terres et al., 1991; Pyzh et al., 1993). Reduced activity of plasminogen activator inhibitor (Tsuruta et al., 1996), the effect on
fibrinolytic system (Liutova et al., 1996), increase in red blood cell deformability and reduced blood viscosity (Green et al., 1991) contribute to the tendency to retard thrombus formation. Although omega-3 fatty acids increase bleeding time secondary to all these actions, increased blood loss has hardly been observed in clinical situations, including coronary bypass surgery (Eritsland et al., 1995). The antithrombotic effects represent additional benefits for coronary heart diseased patients.

**n-3 fatty acids and hypertension:** Investigations in animal models reported both hypotensive and hypertensive responses to oils suggesting a possibility of variation as a function of species and/or model of hypertension, or of the dose or composition of the fish oils which were administered (Kenny et al., 1990; Wynsen et al., 1987). There was also the possibility that the response might vary as a function of saturated fat intake and resting blood pressure (Karanja et al., 1989). Several studies reported a hypotensive effect of fish oils or fish consumption in man (Norris et al., 1986; Loreuz et al., 1983).

**Clinical studies of n-3 fatty acids in vascular disease:** High levels of consumption of n-3 fatty acids from predominantly fish and marine mammal diet resulted in low levels of triglycerides and cholesterol, rather prolonged bleeding times, high levels of n-3 fatty acids in platelet membranes (Dyerberg et al., 1979; Bang et al., 1976). Hirai et al., noted significant rise in n-3/n-6 EPA/AA ratios, inhibition of platelet aggregation and elevation of serum levels of EPA and DHA in populations consuming fish which is equivalent to 2.5 g of EPA (Hirai et al., 1980). Daily intake of 3.5 gm of EPA by patients with
ischaemic disease increased platelet survival, decreased platelet number, platelet factor IV and β-thromboglobulin (Hay et al., 1982).

Russian researchers have studied diets containing different ratios of n-6 to n-3 PUFAs for their hypolipidemic, antiatherogenic, thrombolytic and antihypertensive effects in patients with CHD, familial hyperlipoproteinemia and essential hypertension. Optimizing the n-6 to n-3 PUFA ratio in the diet of patients with cardiovascular disease, by supplementation with fish oil, was found to promote therapeutic effects (Pogozheva et al., 1996).

Fish oil may be an antidote for the cardiovascular risks of smoking (McCarty, 1996; Rodriguez et al., 1996). Intake of fish oil may also be beneficial as an adjuvant treatment in congestive heart failure (McCarty, 1996).

**Miscellaneous effects:** Fish oils or n-3 PUFAs have been investigated in a number of other disorders. Nephroprotective action has been reported in animal models of adriamycin induced (Washiq et al., 1993) and gentamycin induced (Grauer et al., 1996) renal toxicity. Reduced risk of graft rejection along with improved cyclosporine pharmacokinetics has been documented in renal transplant recipients undergoing cyclosporine therapy (Homan et al., 19; Busnach et al., 1998). In ulcerative colitis, a modest corticosteroid sparing effect is noted in active disease (Stenson et al., 1992) and early reactivation of quiescent disease may be prevented (Loeschke et al., 1996). Omega-3-fatty acids restrict hypertriglycerideremia secondary to systemic retinoid therapy (Frati et al., 1994). Anti inflammatory effects have also been observed in cystic fibrosis (Lawrence et al., 1993) and experimental gingivitis (Campan et al.,
Chart 1 Potential mechanisms of action of the major n-3 polyunsaturated fatty acids, EPA and DHA in fish oils

Modification of lipid profile through:
- Inhibitory effect on intestinal cholesterol absorption
- Inhibitory effect on hepatic cholesterol biosynthesis
- Alteration of the clearance of lipoproteins from circulation
- Stimulatory effect on hepatic bile secretion.

Mechanisms of protection against ischemia-reperfusion related cardiac injury:
- Prevention of rapid accumulation of intracellular Ca^{2+} following ischemia by:
  - Inhibiting sarcolemmal ion channels
  - Modulation of Ca^{2+}-current through slow L-type Ca^{2+}-channels
  - Improving the activity of the Ca^{2+}-pump enzyme, Ca^{2+}-Mg^{2+}-ATPase, in the ischemic myocardium
- Electrophysiologic stabilization of cardiac myocytes by blockade of fast voltage-gated sodium channels
- Modulation of cell signaling by other intracellular second messengers-inhibition of reperfusion induced rise in intracellular inositol triphosphate or cyclic adenosine monophosphate levels
- Facilitation of the recovery of mitochondrial energy metabolism
- Altered platelet function
- Inhibition of neutrophil infiltration into ischemic myocardium.

Modification of membrane lipid composition leading to improved functioning of:
- Platelets
- Endothelial cells
- Erythrocytes

Favorable effects on the oxidant-antioxidant balance:
- Augmentation of antioxidant defenses
- Attenuation of free radical generation and lipid peroxidation

Anti-inflammatory and immunoregulatory effects:
- Alteration of the balance of pro- and anti-inflammatory cytokines by competing with other unsaturated fatty acids in eicosanoid metabolism pathways
- Modulation of adhesion molecule expression
- Modulation of neutrophil function
- Modulation of cell-mediated cytotoxicity.
1996). Antimalarial property of n-3 fatty acids has been reported by Kumaratilake et al (1992). Beneficial effects of EPA and DHA in breast cancer (Noguchi et al., 1995; Craig-Schmidt et al., 1993; Shao et al., 1995) and lung cancer were also reported (Abbott et al., 1994).

**Mechanism of action**

Unlike many other pharmacological agents, which have one or few well-defined mechanisms of action, the activity of n-3 PUFA is multifaceted (Chart 1).

**Dosage and administration**

A commonly recommended dose for commercially available fish oil preparations is 10 g daily, taken in the form of soft gelatin capsules or liquid (emulsion) in two divided portions with food (Avijit Hazra et al., 1999). The current preparations are enriched in EPA and DHA to the extent of around 30% (wt/wt) in total and undesirable contents like cholesterol and excess of vitamins A and D are restricted (Avijit Hazra et al., 1999).

Administration of fish oil preparation with food helps to reduce nausea and eructations.

To optimize clinical benefits, appropriate dietary modifications should always be encouraged concurrently with the use of fish oil or fish oil derived n-3 PUFAs. Such a plan should include restriction of total dietary fat and
cholesterol intake and an increase in the ratio of polyunsaturated to saturated fat.

Adverse drug reactions cautions and contra indications: Omega 3 fatty acids are well tolerated. Nausea and eruption appear to be the most common compliant, particularly at high doses (Avijit Hazra et al., 1999). High dose purified n-3 PUFAs also cause diarrhea (Henderson et al., 1994). However the earlier fears of aggravated bleeding tendency (Clarke et al., 1990), and deterioration of glycemic control in diabetic patients (Vessby et al., 1990) have turned out to be unfounded (Eritsland et al., 1995). There are isolated reports of perilesional eczema following application of fish oil to psoriatic plaques (Henneicke-Von Zepelin et al., 1993) elevation of hepatic enzymes (Eritsland et al., 1995) and fever with lymphadenopathy in elderly subjects (Ogden et al., 19 ). Most studies, however, report good to excellent tolerance and hence satisfactory patient compliance.

No absolute contraindication to the use of fish oil or fish oil derived n-3 PUFA is known, though caution has been advised in hemorrhagic disorders, in subjects receiving anticoagulants and in individuals sensitive to aspirin (Avijit Hazra et al., 1999). It would also be prudent to avoid use in children or during pregnancy and breast feeding as there is no experience in these situations.