Indian Journal of Clinical Biochemistry 2000, 15(Suppl.),20-30

HYPERHOMOCYSTEINEMIA AND CARDIOVASCULAR DISEASE: THE NUTRITIONAL PERSPECTIVES

Rajesh Pandey, Seema Gupta, Harbans Lal, H.C.Mehta and S.K. Aggarwal

Department of Biochemistry, Pt. B.D.Sharma, Post Graduate Institute of Medical Sciences, Rohtak (Haryana)

ABSTRACT

Several members of the vitamin B - complex family are known to participate in the normal metabolism of homocysteine (Hcy). Leaving aside the genetic determinants of hyperhomocysteinemia (HHC), the deficiencies of these vitamins can also result in HHC. The situation of sustained and long standing HHC is likely to be prevalent in population groups with low/average socio-economic status, geriatric population and alcohol abusers. If not corrected by supplementation, these population groups certainly are more vulnerable to develop atherosclerosis (AS) and subsequently, cardiovascular disease (CVD). Hyperhomocysteinemia per se and/or HHC- induced oxidative stress result(s) in chronic chemical endothelial injury/ dysfunction, smooth muscle proliferation, prothrombotic state and oxidation of low density lipoproteins (LDL) leading to diverse cardiovascular complications. In the first decade of the new millennium, major research efforts would be directed towards understanding the basic mechanism of HHC-induced oxidative stress and the pathophysiology of HHC-induced CVD, culminating in the evolution of hitherto unknown therapeutic strategies such as nutriceuticals and oxidant-antidotes.

KEY WORDS: Hyperhomocysteinemia; oxidative stress; atherosclerosis; cardiovascular disease.

Cardiovascular disease (CVD) is expected to become the major cause of death worldwide in the 21st century(1). The term CVD encompasses various clinical conditions such as angina pectoris, acute myocardial infarction (AMI), arterial aneurysms, peripheral vascular disease and cerebrovascular disease. However, there is a central pathogenic mechanism behind CVD, i.e. atherosclerosis (AS), the 'root of all evils'. Various constitutional as well as acquired risk factors, in isolation or (more commonly) in combination, interact to create a proatherogenic microenvironment (Fig.1)(1-2).

Several theories (response to injury; monoclonal; clonal - senescence; encrustation; lipogenic; and lysosomal) have been put forward to explain the pathogenic mechanism of AS, of which the *'response-to-injury'* hypothesis as proposed by Ross and Glomset seems to be more plausible(3). It suggests that either endothelial injury and/or dysfunctional endothelial responses to pathogenic stimuli is the key initiating event in AS. Hypercholesterolemia, tobacco smoke-bye products, glycosylation products secondary to diabetes mellitus or ageing, altered biomechanical forces as well as infections are some of the traditional, potentially injurious atherogenic agents.

Hyperhomocysteinemia (HHC) is a newly identified, independent, constitutional and/or potentially modifiable acquired risk factor associated with premature CVD. Ironically, when McCully and Wilson (4) proposed the *homocysteine-theory* of AS in 1975, it attracted very little attention for nearly

Author for correspondence
Dr. S.K. Aggarwal, Shanti Sadan, 6 - Gole Market, Model Town, Rohtak-124001

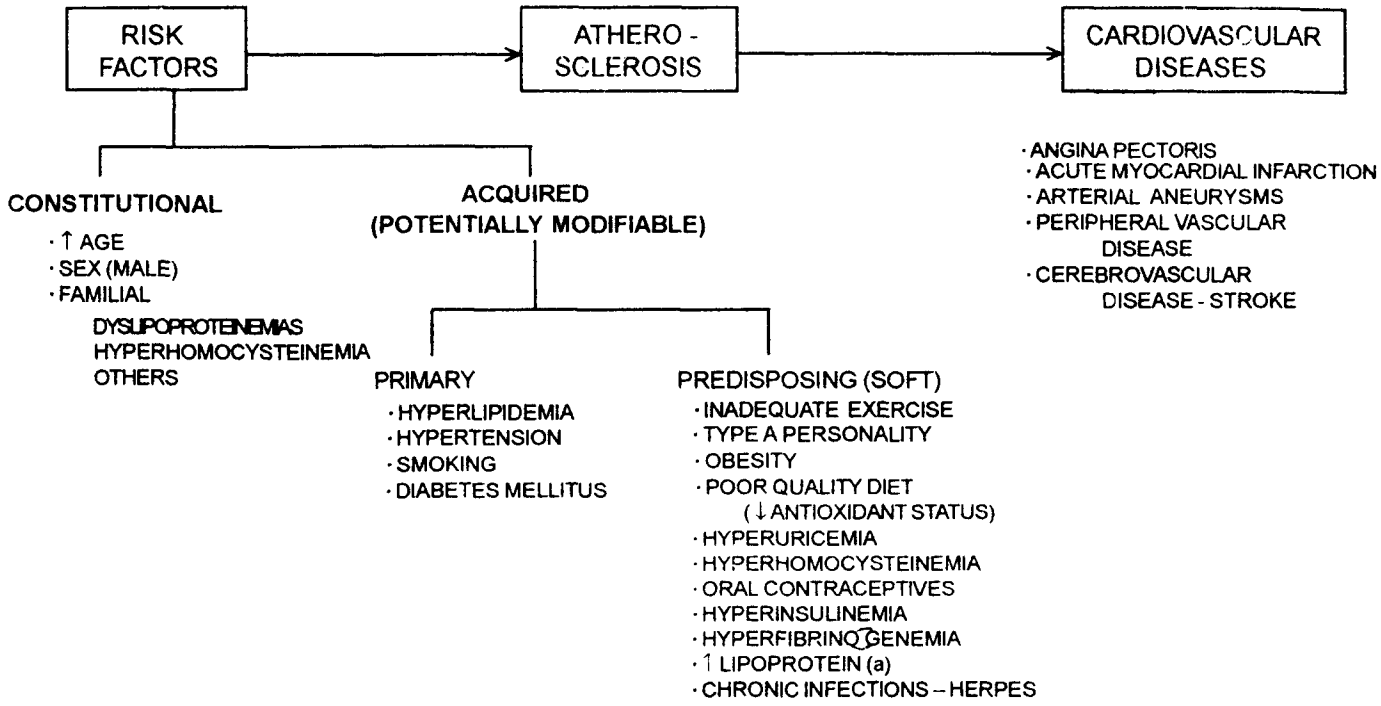

Fig. 1. Risk factors - Atherosclerosis and cardiovascular diseases

two decades. Only recently, HHC has been linked with coronary artery disease, peripheral vascular disease, cerebrovascular disease as well as recurrent venous thrombosis and has been aptly labelled as the 'new cholesterol' (5-9). Though HHC has also been shown to be associated with neural tube defects, Alzheimer's disease and osteoporosis (10-12), the present review restricts to highlight the link between HHC and CVD, as well as the possible nutritional implications.

Homocysteine metabolism and determinants of hyperhomocysteinemia

Dietary methionine (Met), a sulphur-containing essential amino acid, is the sole source of homocysteine (Hcy) in the body. Normally, Met is 'activated' to S-adenosyl methionine (SAM) which serves as the precursor for the synthesis of polyamines, compounds essential for the regulation of cell growth as well as ion transport(13). More importantly, SAM follows the transmethylation pathway resulting in the formation of S-adenosyl homocysteine (SAH) and a variety of methylated derivatives of considerable biological significance. The subsequent hydrolysis of SAH yields Hcy and adenosine.

The methionine is regenerated by the transfer of a methyl group to Hcy from N^5-methyl-tetrahydrofolic acid (N^5-CH_3-THFA) in the reaction catalyzed by methylcobalamine N^5-CH_3-THFA : homocysteine methyl transferase (MTHMT), also called methionine synthase (MS). Actually, SAM initiates the reaction whereas N^5-CH_3-THFA serves as the methyl donor for the synthesis of Met, completing the activated 'methyl cycle'. The latter is intimately linked with the 'folate cycle' and the enzymes of both these pathways are widely distributed. In addition, Hcy may be converted to Met in a reaction catalyzed by betaine : homocysteine methyl transferase (BHMT), which is, however, limited only to the liver and kidney(9-14).

Under conditions of Met excess or during an increased cellular demand for cysteine (Cys), the bulk of Hcy is channelled into the 'transsulphuration pathway'. This route is confined only to the liver,

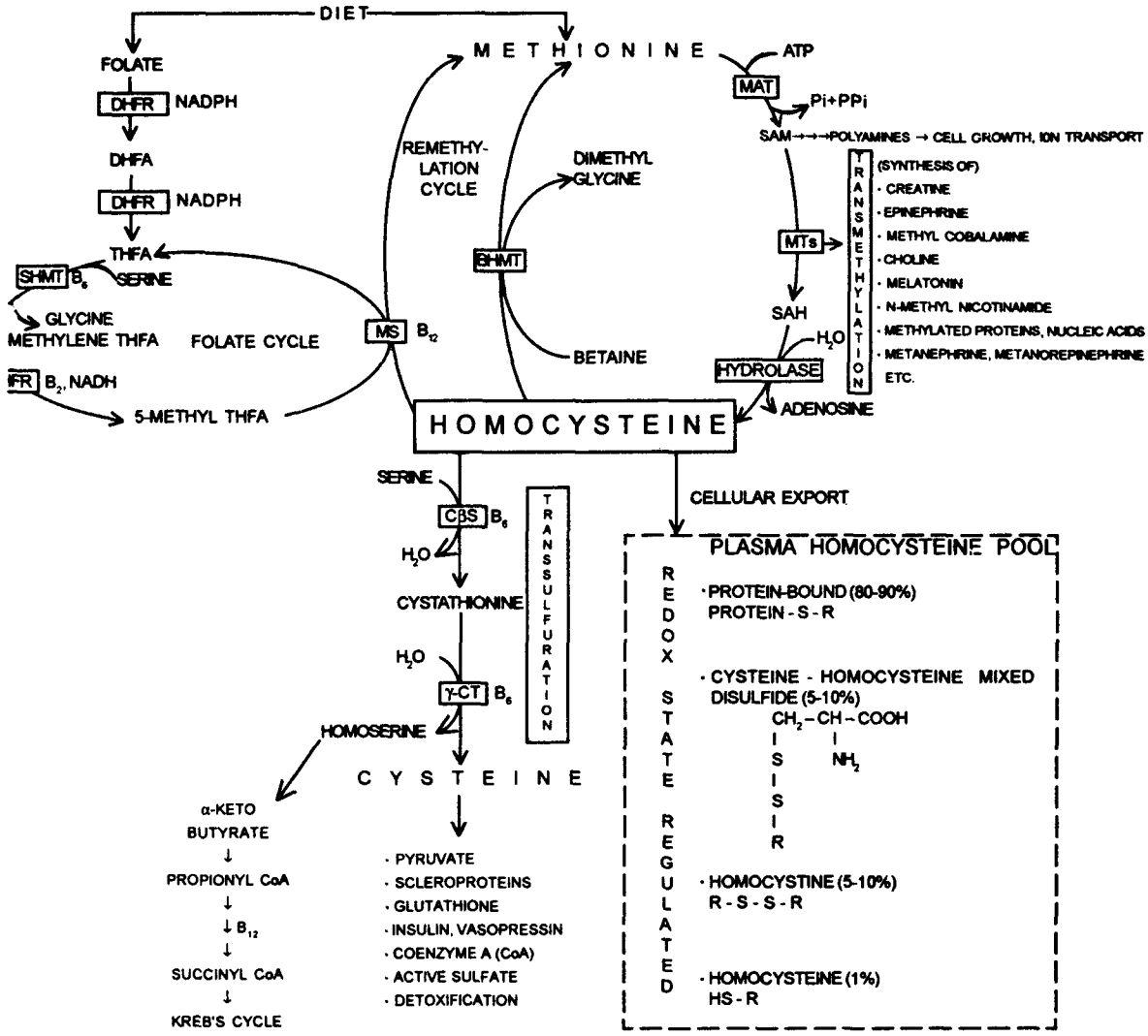

Fig. 2 : Homocysteine metabolism.

R = $-CH_2 - CH_2 - CH - COOH$
NH_2

kidney, pancreas and brain. In the first step, Hcy is converted to cystathionine catalyzed by the rate-limiting enzyme cystathionine-β-synthase (CβS). Subsequently, cystathionine is converted to Cys by the action of γ-cystathionase (γ-CT). Both these enzymes are vitamin B_6 - dependent. Cysteine, a non-essential sulphur-containing amino acid, can now participate in any of its multifacet functions, as per the cell's need. It is, therefore, clear that Hcy lies at the intersection of the remethylation and transsulphuration pathways (Fig. 2)(15,16).

Intracellular Hcy concentration is maintained at a low level by a specific cellular export mechanism(17). The total plasma Hcy pool comprises of the protein bound form, mixed disulphides, homocystine and free Hcy. The protein -bound form represents the predominant circulating component whereas the contribution of free Hcy to it, is minimal. However, the total Hcy pool is a mobile one as each of its component is freely exchangeable, depending on the redox state(18,19).

Several vitamins, either as second substrates or as prosthetic groups, are essential in Hcy metabolism. Although some of them directly participate in the remethylation and/or transsulphuration pathways, others are indirectly linked through the enzymes involved in the 'folate cycle'. It is reasonable to argue that besides a disturbance in the Hcy metabolism itself, any abnormality in the metabolic pathway of the individual cofactors, could also be an important determinant of HHC. Such an abnormality may lie at the level of the quality of food, digestion, absorption or enzymatic bioactivation of cofactors. Some aberrations in vitamin B_{12} (cobalamine, cbl) metabolism associated with HHC illustrate this aspect - (i) cblC and cblD : deficiency of a reductase needed for the formation of methyl cobalamine and hydroxy cobalamine; (ii) cblE and cblG : impaired activity of a particular component required to maintain a reduced form of cobalamine on the methyl transferase apoenzyme; and (iii) cblF : an impaired lysosomal efflux of cbl (20).

Various physiological as well as pathological factors may contribute to HHC (Fig.3). Until recently, a genetic deficiency and/or a mutation in several important enzymes such as CβS, MS or MTHFR, were the only known causes of HHC. Extensive research, however, has enabled scientists to recognize a host of other causes, as well. Some of them are attributable to a poor nutritional status, various disease conditions or certain drugs(21-33). The three most important conditions leading to HHC can be summarized as - (i) genetic deficiencies of Hcy-metabolizing enzymes; (ii) a deficiency of folate and/or vitamin B_{12} and/or vitamin B_6; and (iii) chronic renal failure(34).

Hyperhomocysteinemia-oxidative stress-atherosclerosis-cardiovascular disease

Hyperhomocysteinemia promotes AS chiefly by causing-

(i) endothelial cell (EC) dysfunction; (ii) smooth muscle cell (SMC) proliferation; (iii) disturbance in the coagulation system; and (iv) oxidation of low density lipoproteins (LDL), forming oxidized LDL(ox-LDL), a molecule endowed with marked proatherogenic characteristics (16, 35). However, the aforesaid dysfunctions are also attributable to oxidative stress per se which is thought to underlie most of the basic pathogenic mechanisms put forward for AS(36). Hence, it is reasonable to propose that the deleterious effects of HHC on the cardiovascular system could be mediated by oxidative stress (Fig. 4).

Reactive oxygen species (ROSs, a term encompassing oxygen free radicals as well as some non-radicals) are constantly formed in the human body as the obligatory products of aerobic respiration. Because they are capable of inducing damage to cellular proteins, nucleic acids and biological membranes (if present in pathophysiological concentrations), evolution has endowed the body with a hierarchy of protective mechanisms, collectively termed as the antioxidant defense system (AoDS). A pathological imbalance has been termed as oxidative stress which may make an important contribution to disease pathology (37). Alternatively, increased oxidative stess may also be a result of the disease itself (38,39).

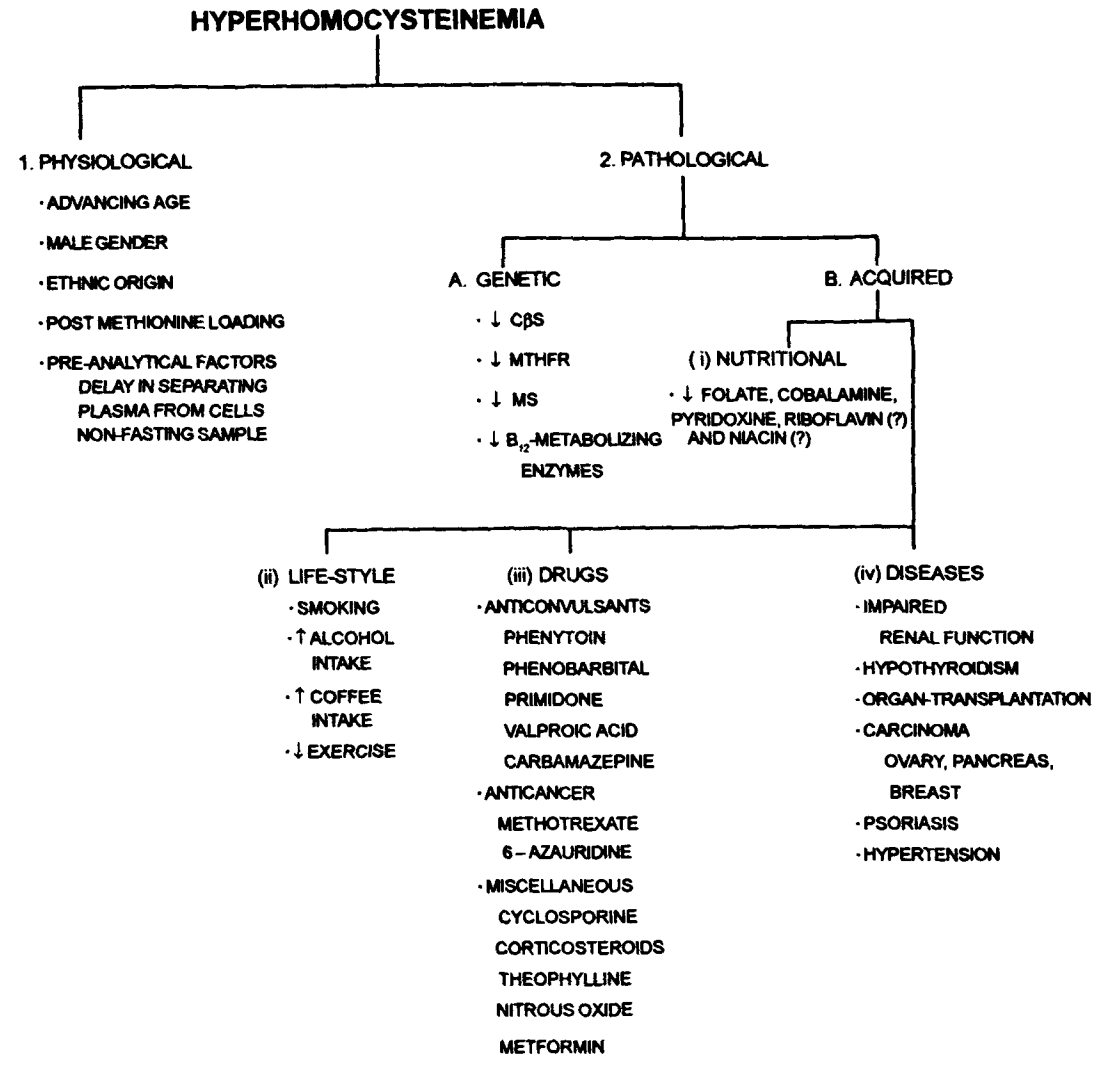

Fig. 3 : Determinants of hyperhomocysteinemia.

Studies have shown that auto-oxidation of Hcy produces ROSs, specially in the presence of free transition metal ions (tM_n^{++}). Homocysteine also contributes to the reduction of cystine to cysteine. In turn, the latter can not only be auto-oxidized to yield $O_2^{\cdot -}$ and H_2O_2, but also helps to oxidize glutathione (GSH) in the presence of tM_n^{++} (40,41). Moreover, the ROSs produced as a result of auto-oxidation of Hcy and/or Cys could be responsible for the mobilization of iron from the cellular stores. It is known that free iron is capable of exerting toxic effects through its ability to catalyze the formation of highly reactive hydroxyl radicals (OH)$^{\cdot}$ via Fenton and Haber-Weiss reactions (42).

Since GSH is known as the 'redox battery' of the cell, any of the following factors leading to its depletion can profoundly affect the intracellular redox status - (i) GSH oxidation in the presence of Cys and tM_n^{++}; (ii) GSH consumption in the process of detoxification of ROSs ; (iii) formation of Hcy-GSH mixed disulphide; and (iv) reduced Cys availability (GSH precursor) in cases of impaired transsulphuration pathway. Concomitantly, if there is a deficiency of other critical antioxidants, eg. vitamin C, vitamin E and β-carotene (the 'big three' antioxidants), the net result is an increased oxidant burden along with a reduced efficiency of the AoDS. This is supported by the observation that patients with sickle cell disease are deficient in vitamin C as

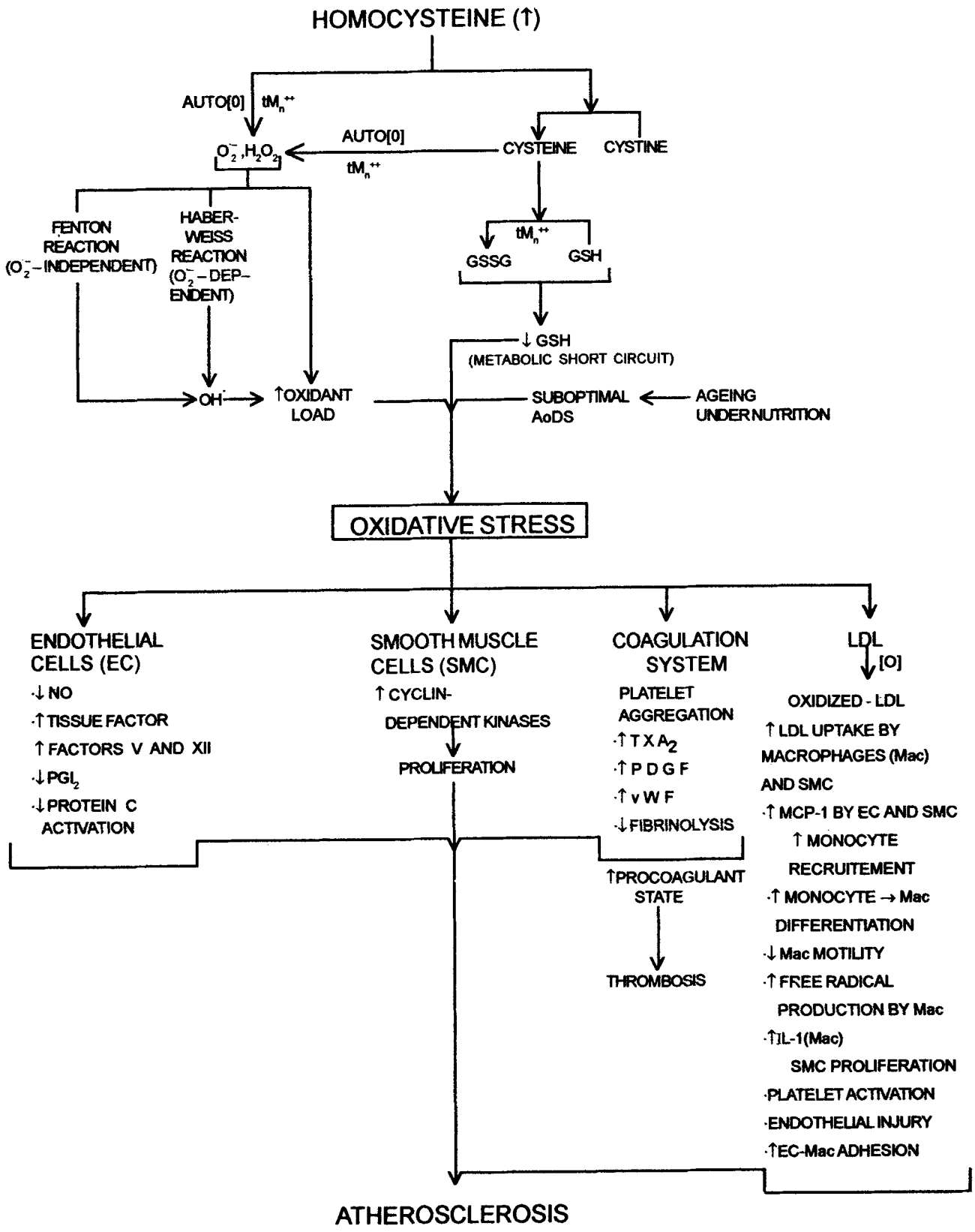

Fig. 4 : Hyperhomocysteinemia and atherosclerosis. [O] Denotes oxidation

well as glutathione and are more vulnerable to oxidant damage (43). Other factors such as advancing age, suboptimal nutrition, co-existing diseases etc. may independently serve to further compromise the AoDS(44-45).

The ROSs - induced endothelial injury may be important in the pathophysiology of AS. The injury may alter the functional characteristics of the endothelium such as its role as a permeability barrier, its nonthrombogenic character, its ability to release growth factors and vasoactive substances such as nitric oxide (NO), prostacyclin (PGI_2), thromboxane A_2 (TXA_2) and endothelin-1, as well as its regeneration capacity. At the other extreme, endothelial injury may lead to EC dysfunction and endothelial retraction, exposing the underlying connective tissue or accumulated foam cells, such as macrophages that form the first and the ubiquitous lesions of AS, the fatty streak(46,47).

Endothelial dysfunction apears to have immediate detrimental consequences as well as adverse long term effects, including vascular remodeling. Endothelial dysfunction is associated with impaired tissue perfusion particularly during stress and paradoxical vasoconstriction of large conduit vessels including the coronary arteries. These effects may cause or contribute to myocardial ischemia(48). Several mechanisms may be involved in the development of endothelial dysfunction such as reduced synthesis and/or release of NO and enhanced oxidative inactivation of NO after its release from EC by radicals, ox - LDL and high levels of Hcy (46,49).

The ability of ROSs to alter EC function depends on their concentration, duration of exposure, the antioxidant status and their interaction with other bio-active compounds such as growth factors, cytokines, kinins, eicosanoids, aminoacids, nucleosides etc.(50-53) The mechanism of oxidant-induced EC dysfunction, though incompletely understood, may be due to the oxidant induced rise in intracellular ionic calcium ($[Ca^{2+}]_i$) by various mechanisms. This rise in $[Ca^{2+}]_i$ either alone or in combination with ROSs, is known to initiate a cascade of cellular events such as the activation of phospholipases (PLA_2, PLC, PLD), protein kinases, endonucleases, proteases, expression of several immediate - early genes and last but not the least, the triggering of Ca^{2+} - influx possibly via the release and/or phosphorylation of a specific calcium influx factor (52,53). Collectively, these events lead to altered EC responses to agonists, enhanced permeability, increase in the prothrombotic mediators and reduced NO bio-availability, thereby jeopardizing the very survival of the cell.

A subnormal concentration of NO in the vascular microenvironment critically hampers the regulation of vasomotor tone. Moreover, NO is also important in modulating the local Hcy concentration as it forms a NO-Hcy adduct, thereby acting as a Hcy-buffer(16). Thus, a low NO allows Hcy to exert its multiple deleterious effects. Certainly, the HHC-induced oxidative stress seems not only to initiate but also to perpetuate endothelial damage, establishing a vicious cycle of EC-dysfunction.

The ROSs and tM_n^{++} dependent formation of ox-LDL facilitates the recruitment of blood monocytes into the arterial wall, their differentiation into macrophages (Mac) and the subsequent Mac activation. It also promotes the procoagulant state, smooth muscle cell (SMC) proliferation and EC apoptosis (1,54-58). Further, ox-LDL activates a protein called peroxisome proliferator activated receptor-γ-which enhances the transcription of the scavenger receptor gene, the protein product of which is CD36 that, in turn, mediates ox-LDL uptake into Mac, thus establishing a vicious cycle(59).

Homocysteine leads to a prothrombotic state as a result of endothelial dysfunction at a variety of levels. It promotes platelet aggregation as well as the accumulation of thrombogenic molecules such as TXA_2 and vonWillebrand's factor, besides inhibiting fibrinolysis (60-62). Alongwith platelet derived growth factor, it promotes SMC proliferation. These effects on the coagulation system and SMC are due to the activation of critical transcription factors such as Nuclear Factor – Kappa B (NF-κB) by ROSs. The activation of NF-κB triggers the expression of cell adhesion molecules thereby contributing to the proliferative effects of Hcy on the vascular wall(63).

Future Perspectives

The above discussion brings out the fact that the persistently raised level of Hcy is conducive for the development of oxidative stress. The latter, in turn, favours the pathogenesis of AS, the 'culprit' behind various forms of CVD. Conceivably, the concomitant presence of other risk factor(s) can amplify the entire scenario of AS and its sequelae. From the therapeutic point of view, two distinct facets emerge from the *hyperhomocysteinemia-oxidative stress-atherosclerosis* (HOSAS) hypothesis. Firstly, the limited availability of one or more co-factors such as folic acid, vitamin B_{12}, B_6, B_2(?) and niacin(?)play an important role in the pathogenesis of HHC. Secondly, once HHC is established, the antioxidant status of the individual becomes the central deciding factor as far as the development of AS is concerned. The antioxidant micronutrients especially β-carotene, vitamin E and vitamin C, if present in adequate concentrations, may counter the oxidant challenge.

The micronutrients known to be involved in the normal metabolism of Hcy, if required, can be used in supplemental form. For instance, some cases of HHC respond to vitamin B_6 supplemenation due to enhanced CβS activity, in whom there is either a decreased affinity for the co-factor or an increased degradation of the mutant enzyme(20). However, the supplementation of antioxidant vitamins (the 'big three') needs to be practiced with caution since vitamin C in megadoses is known to behave paradoxically as a pro-oxidant, in the presence of tM_n^{++}, leading to the inactivation of vitamin B_{12} and/ or intrinsic factor thereby compromising the role of folic acid in Hcy metabolism (64). Till clinical trials evaluate the role of vitamins to debridge the HHC-CVD bond and establish Hcy as a biomarker for the nutritional status a 'high nutriceutical diet' should be an integral part of the physician's prescription (65,66).

REFERENCES

1. Basha, B.J. and Sowers, J.R. (1995) Atherosclerosis : an update. Amer. Heart J. 131, 1192-1202.
2. Robinson, K. and Loscalzo, J. (1998) Other risk factors for coronary artery disease : homocysteine, lipoprotein (a), fibrinogen, and plasminogen activator inhibitor. In : Textbook of Cardiovascular Medicine, Ed. Topol, E.J. Lippincott-Raven, Philadelphia, p 231-247.
3. Ross, R. and Glomset, J.A. (1973) Atherosclerosis and the arterial smooth muscle cell : proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. Science 180, 1332-1339.
4. McCully, K.S. and Wilson, R.B. (1975) Homocysteine theory of arteriosclerosis. Atherosclerosis 22, 215-227.
5. Genest, J.J. Jr., McNamara, J.R., Salem, D.N., Wilson, P.W.F., Schaefer, E.J. and Malinow, M.R. (1990) Plasma homocyst(e)ine levels in men with premature coronary artery disease. J. Amer. Coll. Cardiol. 16, 1114-1119.
6. Taylor, L.M. Jr. , Defrang, R.D., Harris, E., Jr. and Porter, J.M. (1991). The association of elevated plasma homocyst(e)ine with progression of symptomatic peripheral arterial disease. J. Vasc. Surg. 13, 128-136.
7. Brattstrom, L., Lindgren, A., Israelsson, B., Malinow, M.R., Norrving, B., Upson, B. and Hamfelt, A (1992) Hyperhomocysteinemia in stroke : prevalence, cause, and relationships to type of stroke and stroke risk factors. Eur. J. Clin. Invest. 22, 214-221.
8. den Heijer, M., Blom, H.J., Gerrits, W.B.J., Rosendaal, F.R., Haak, H.L., Wijermans, P.W. and Bos, G.M.J. (1995) Is hyperhomocysteinemia a risk factor for recurrent venous thrombosis? Lancet 345, 882-885.
9. Jacobsen, D.W. (1998) Homocysteine and vitamins in cardiovascular disease. Clin. Chem. 44, 1833-1843.

10. Mills, J.L., McPartlin, J.M., Kirke, P.N., Lee, Y.J., Conley, M.R., Weir, D.G and Scott, J.M. (1995) Homocysteine metabolism in pregnancies complicated by neural tube defects. Lancet 345, 149-151.
11. Miller, J.W.(1999) Homocysteine and Alzheimer's disease. Nutr. Rev. 57, 126-129.
12. Jackson, S.H. (1973) The reaction of homocysteine with aldehyde: an explanation of the collagen defects in homocystinuria. Clin. Chim. Acta 45, 215-217.
13. Williams, K. (1997) Modulation and block of ion channels : a new biology of polyamines. Cell. Signal. 9, 1-13.
14. Moolenar, S.H., Poggi-Bach, Jo., Engelke, U.F.H., Corstiaensen, J.M.B., Heerschap, A., De Jong, J.G.N., Binzak, B.A., Vockley, J. and Wevers, R.A. (1999) Defect in dimethylglycine dehydrogenase, a new inborn error of metabolism : NMR spectroscopy study. Clin. Chem. 45, 459-464.
15. Selhub, J. and D'Angelo, A. (1998) Relationship between homocysteine and thrombotic disease. Amer. J. Med. Sci. 316, 129-141.
16. Sethi, K.K. and Mantri, R.R. (1999) Homocysteine - emerging evidence as an independent risk factor for coronary artery disease (editorial). Cardiol. Today III, 121-129.
17. Christensen, B., Refsum, H., Vintermyr, O. and Ueland, P.M. (1991) Homocysteine export from cells cultured in the presence of physiological or superfluous levels of methionine : methionine loading of nontransformed, transformed, proliferating, and quiescent cells in culture. J. Cell Biol. 146, 52-62.
18. Ueland, P.M. (1995) Homocysteine species as components of plasma redox thiol status. Clin. Chem. 41, 340-342.
19. Mansoor, M.A., Svardal, A.M., Schneede, J. and Ueland, P.M. (1992) Dynamic relation between reduced, oxidized and protein-bound homocysteine and other thiol components in plasma during methionine loading in healthy men. Clin. Chem. 38, 1316-1321.
20. Elsas, L.J., Longo, N. and Rosenberg, L.E. (1998) Inherited disorders of amino acid metabolism and storage. In : Harrison's Principles of Internal Medicine, Eds. Fauci, A.S., Braunwald, E., Isselbacher, K.J., Wilson, J.D., Martin, J.B., Kasper, D.L., Hauser, S.L. and Longo, D.L. 14th edn., McGraw-Hill, New York, p. 2194-2203.
21. Dudman, N.P.B., Wilcken, D.E.L., Wang, J., Lynch, J.F., Macey, D. and Lundberg, P. (1993) Disordered methionine/homocysteine metabolism in premature vascular disease : its occurrence, cofactor therapy, and enzymology. Arterioscler. Thromb. 13, 1253-1260.
22. Ubbink, J.B., Vermaak, W.J.H., van der Merwe, A. and Becker, P.J. (1993) Vitamin B_{12}, vitamin B_6 and folate nutritional status in men with hyperhomocysteinemia. Amer. J. Clin. Nutr. 57, 47-53.
23. Hultberg, B., Berglund, M., Andersson, A. and Frank, A. (1993) Elevated plasma homocysteine in alcoholics. Alcohol Clin. Exp. Res. 17, 687-689.
24. Goyette, P., Frosst, P., Rosenblatt, D.S. and Rozen, R. (1995) Seven novel mutations in the methylenetetrahydrofolate reductase gene and genotype/phenotype correlations in severe methylenetetrahydrofolate reductase deficiency. Amer. J. Hum. Genet. 56, 1052-1059.
25. Rozen, R. (1996) Molecular genetic aspects of hyperhomocysteinemia and its relation to folic acid. Clin. Invest. Med. 19, 171-178.
26. Scott, J. and Weir, D. (1996) Homocysteine and cardiovascular disease (editorial). Quart. J. Med. 89, 561-563.
27. Harmon, D.L., Woodside, J.V., Yarnell, J.W.G., McMaster, D., Young, I.S., McCrum, E.E., Gey, K.F., Whitehead, A.S. and Evans, A.E. (1996) The common 'thermolabile' variant of methylene tetrahydrofolate reductase is a major determinant of mild hyperhomocysteinemia. Quart. J. Med. 89, 571-577.
28. Tsai, M.Y., Bignell, M., Schwichtenberg, K. and Hanson, N.Q. (1996) High prevalence of a mutation in the cystathionine β-synthase gene. Amer. J. Hum. Genet. 59, 1262-1267.

29. Arnadottir, M., Hultberg, B., Vladov, V., Nilsson-Ehle, P. and Thysell, H. (1996) Hyperhomocysteinemia in cyclosporin-treated renal transplant recipients. Transplantation 61, 509-512.
30. Chen, L.H., Liu, M.L., Hwang, H.Y., Chen, L.S., Korenberg, J and Shane, B. (1997) Human methionine synthase - cDNA cloning, gene localization and expression. J. Biol. Chem. 272, 3628-3634.
31. Miner, S.E.S., Evrovski, J. and Cole, D.E.C. (1997) Clinical chemistry and molecular biology of homocysteine metabolism : an update. Clin. Biochem. 30, 189-201.
32. Jacobsen, D.W. (1998) Acquired hyperhomocysteinemia in heart transplant recipients (editorial). Clin. Chem. 44, 2238-2239.
33. Willams, R.H. and Maggiore, J.A. (1999) Hyperhomocysteinemia. Pathogenesis, clinical significance, laboratory assessment and treatment. Lab. Med. 30, 468-475.
34. Guldenner, C.V., Janssen, J.F.M., De Meer, K., Donker, A.J.M. and Stehouwer, C.D.A. (1999) Effect of folic acid and betaine on fasting and post methionine-loading plasma homocysteine and methionine levels in chronic hemodialysis patients. J. Int. Med. 245, 175-183.
35. Naito, M., Hayashi, T. and Iguchi, A. (1995) New aproaches to the prevention of atherosclerosis. Drugs 50, 440-453.
36. Witzhum, J.L. (1994) The oxidation hypothesis of atherosclerosis. Lancet 344, 793-795.
37. Lal, H., Pandey, R. and Aggarwal, S.K. (1999) Antioxidant vitamins and chemoprevention. Ind. J. Clin. Biochem. 14, 1-11.
38. Lal, G. Yadav, S.P.S., Aggarwal, S.K., Bansal, S., Chugh, K. and Lal H. (1996) Plasma vitamin E status in head and neck cancers. Ind. J. Clin. Biochem. 11, 46-48.
39. Abrol, P., Sharma, N. and Lal, H. (1997) Vitamin E status in protein energy malnutrition. Ind. J. Clin. Biochem. 12, 125-127.
40. Welch, G.N., Upchurch, G.R. and Loscalzo, J. (1997) Homocysteine, oxidative stress, and vascular disease. Hosp. Pract. 32, 81-92.
41. Hogg, N. (1999) The effect of cyst(e)ine on the auto-oxidation of homocysteine. Free Radic. Biol. Med. 27, 28-33.
42. Mateos, F., Brock, J.H. and Perez-Arellano, J.L. (1998) Iron metabolism in the lower respiratory tract. Thorax 53, 594-600.
43. Fakhri, M.E., Aggarwal, S.K., Gayoum, A. and Sheriff, D.S. (1991) Reduced plasma ascorbic acid and red cell glutathione contents in sickle cell disease - a preliminary report. Ind. J. Clin. Biochem. 6, 47-50
44. Halliwell, B. (1999) Establishing the significance and optimal intake of dietary antioxidants : the biomarker concept. Nutr. Rev. 57, 104-113.
45. Lal, H., Chugh, K., Saini, V., Kaur, J. and Saini, A.S. (1992). Effect of ageing on some free radical scavangers and the metabolic consequence in rats. Ind. J. Clin. Biochem. 7,143-146.
46. Meidell, R.S. (1994) Southwestern internal medicine conference : endothelial dysfunction and vascular disease. Amer. J. Med. Sci. 307, 378-389.
47. de Valk-de Roo, G.W., Stehouwer, C.D.A., Lambert, J., Schalkwijk, C.G., van der Mooren, M.J., Kluft, C. and Netelenbos, C. (1999) Plasma homocysteine is weakly correlated with plasma endothelin and von Willebrand factor but not with endothelium-dependent vasodilation in healthy postmenopausal women. Clin. Chem. 45, 1200-1205.
48. Drexler, H. and Hornig, B. (1999) Endothelial dysfunction in human disease. J. Mol. Cell. Cardiol. 31, 51-60.
49. Welch, G.N., Upchurch, G.R., Jr. and Loscalzo, J. (1997) Hyperhomocyst(e)inemia and atherothrombosis. Ann. NY. Acad. Sci. 811, 48-58.
50. Singh, J., Garg, K.N., Garg, D. and Lal, H. (1988). Effect of adenosine and inosine on experimental myocardial infarction in rats. Ind. J. Exp. Biol. 26, 771-774.
51. Singh, J. Garg, K.N., Garg, D., Chugh, K.and Lal, H.(1989). Effect of aspartate and glutamate on experimental myocardial infarction in rats. Ind. J. Exp. Biol. 27, 621-624.

52. Natarajan, V. (1995) Oxidants and signal transduction in vascular endothelium. J. Lab. Clin. Med. 125, 26-37.
53. Trump, B.F. and Berezesky, I.K. (1995) Calcium-mediated cell injury and cell death. FASEB J. 9, 219-228.
54. Cushing, S.D. and Fogelman, A.M. (1992) Monocytes may amplify their recruitement into inflammatory lesions by inducing monocyte chemotactic protein. Arterioscler. Thromb. 12, 78-82.
55. Godfried, S.L. and Deckelbaum, L.I (1995) Natural antioxidants and restenosis after percutaneous transluminal coronary angioplasty (editorial). Amer. Heart J. 129, 203-210.
56. Gambhir, D.S. and Gambhir, J.K. (1997) Oxidised low density lipoprotein, antioxidants and coronary atherosclerosis. Ind. Heart J. 49, 19-22.
57. Heinecke, J.W. (1999) Is lipid peroxidation relevant to atherogenesis? (commentary). J. Clin. Invest. 104, 135-136.
58. Sata, M. and Walsh, K. (1998) Oxidized LDL activates Fas-mediated endothelial cell apoptosis. J. Clin. Invest. 102, 1682-1689.
59. Wolf, G. (1999) The role of oxidized low-density lipoprotein in the activation of peroxisome proliferator-activated receptor γ implications for atherosclerosis. Nutr. Rev. 57, 88-91.
60. Winder, A.F. (1998) Homocysteine and cardiovascular disease (editorial). J. Clin. Pathol. 51, 713.
61. Harpel, P., Zhang, X. and Borth, W. (1996) Homocysteine and hemostasis : pathogenetic mechanisms predisposing to thrombosis. J. Nutr. 126 (Suppl), 1285S-1289S.
62. Guba, S.C., Fink, L.M. and Fonesca, V. (1998) Hyperhomocysteinemia and premature vascular occlusive disease. Amer. J. Med. Sci. 315, 279-285.
63. Price, D.T. and Loscalzo, J. (1999) Cellular adhesion molecules and atherogenesis. Amer. J. Med.107, 85-97.
64. Mix, J.A. (1999) Do megadoses of vitamin C compromise folic acid's role in the metabolism of plasma homocysteine? Nutr. Res. 19, 161-165.
65. Still, R.A. and McDowell, I.F.W. (1998) Clinical implications of plasma homocysteine measurement in cardiovascular disease. J. Clin. Pathol. 51, 183-188.
66. Cooke, J.P. (1998) Nutriceuticals for cardiovascular health. Amer. J. Cardiol. 82 (Suppl), 43S-46S.