FIBRINOLYSIS - ITS RELATIONSHIP TO CORONARY HEART DISEASE AND "RISK FACTORS".

by

Isobel D. Walker.

Submitted for the degree of M.D. in the Faculty of Medicine, University of Glasgow.

This work was performed in the Department of Haematology, Royal Infirmary, Glasgow.

January, 1983.

CONTENTS.

Page

Page

Page

Page

Page

Page

LIST OF TABLES.

Page

ACKNOWLEDGEMENTS.

I wish to acknowledge the help and encouragement I have received from many people in the preparation of this Thesis.

- Dr. J.F. Davidson, Consultant Haematologist, Glasgow Royal Infirmary who helped me design the study, encouraged me to complete it and constructively criticised its presentation.

- Dr. I. Hutton, Senior Lecturer in Cardiology, University of Glasgow whose patients were studied.

- Mrs. F. McCall, Mrs. J. McKenzie, Mrs. R. Robertson & Dr. J.A. Conkie, Thrombosis Research Group, Glasgow Royal Infirmary for their expert technical assistance in the laboratory and for the many hours which they spent talking to patients and volunteers having "venous occlusion tests".

- Dr. G.A. McDonald, Consultant Haematologist, Glasgow Royal Infirmary, who advised me on my Higher Medical Training.

- Prof. H.G. Morgan, Professor of Pathological Biochemistry, Dr. F. Ballantyne, Principal Biochemist and the staff of the Department of Clinical Biochemistry, Glasgow Royal Infirmary, who arranged and who carried out the several hundred plasma lipid profiles needed for this study.

- The Wellcome Trust for a Research Travel Grant which enabled me to visit the Gaubius Institute, Leiden, Netherlands.

- The patients and volunteers who consented willingly and complained seldom.

- Mr. A. McDonach, Centre for Industrial Innovation, University of Strathclyde, who wrote the programmes for the Apple II.

- Mrs. D. Hamilton who typed the manuscript so expertly.

- and my family who tolerated me throughout.

SUMMARY.

This thesis reviews the literature relating to the various factors which are important in the development of clinically significant coronary artery atherosclerosis. The pathogenesis and progress of atherosclerotic lesions are considered and the literature regarding the various so-called coronary heart disease (C.H.D.) "risk factors" examined. The importance of two main non-modifiable "risk factors", age and sex, and three main modifiable "risk factors", cigarette-smoking, hypertension and increased plasma lipid levels, is discussed. A possible relationship between poor plasma fibrinolytic activity and the risk of developing C.H.D. is postulated.

Current knowledge about the various components of the fibrinolytic system is presented and evidence of physiological and pathological variations in fibrinolysis considered.

A standardised procedure for measuring plasma fibrinolytic activity in euglobulin fractions applied to fibrin plates was introduced and details of this procedure are presented along with the results of a number of experiments carried out to examine the effect of varying different aspects of the standardised procedure.

As previously reported by other researchers, plasma fibrinolytic activity has been shown, normally to increase

locally in response to venous occlusion of a limb. A fifteen minute venous occlusion test of an arm was developed and assessed as a means of studying the fibrinolytic response to a challenge. This test has been found to be reproducible and acceptable to both healthy male volunteers and male patients.

In agreement with other workers, plasma fibrinolytic activity, resting and post venous occlusion, has been found to vary in relation to the existence of a number of the C.H.D. "risk factors" - in particular to plasma lipid levels but also, probably, to age and to diastolic blood pressure. No direct relationship between cigarette smoking habit and plasma fibrinolytic activity could be defined. Male subjects only were studied.

The relationships between plasma fibrinolytic activity and C.H.D. "risk factors" were present both in healthy controls and in men known to have C.H.D.

Results are presented which suggest that C.H.D. itself is associated with reduced plasma fibrinolytic activity at rest and, possibly, post venous occlusion. This impaired fibrinolytic activity appears to be independent of co-existing C.H.D. "risk factors".

It is not possible, from the evidence obtained in this study, to comment on whether impaired plasma fibrinolytic activity is an important factor contributing to the risk of developing C.H.D. or is a secondary effect of the disease.

PROLOGUE.

Without question coronary heart disease (C.H.D.) is the major cause of death in middle and old age in the United Kingdom. In men aged 45 - 54 years, cardiovascular disease is responsible for about 50% of all deaths and more than three quarters of these deaths are due to C.H.D. (Joint Working Party report - 1976). These figures are perhaps even more striking when it is realised that probably the most emotive scourge of modern medicine, cancer, claims a mere 26% of all deaths in this age group. Within the United Kingdom, the highest rates for ischaemic heart disease and cerebrovascular disease mortality are in the North and West, the worst region being West Central Scotland (Fulton et al 1978). The problem of the very high mortality from C.H.D. in Scotland as a whole was further emphasised by figures from the World Health Statistics Annual report for 1972 (World Health Statistics Annual) which indicated that Scotland was second only to Finland in having the highest rates in the world for men aged 35 - 64. Even so, death rates tend to underestimate the real size of the problem. Many people develop C.H.D. which may be severe and even disabling, yet they die of other causes.

The precise relationships between clinically observed end-stage events of C.H.D. - myocardial infarction, cardiac failure, arrythmias or sudden death - and coronary

atherosclerosis are not understood. That atherosclerosis is the cause of occlusive arterial disease is, of course, a widely held view, but whilst it is certainly true that a close positive correlation does exist between the incidence of atheroma and C.H.D. death rates in many countries, there are also some very striking disassociations (Morris and Crawford 1958; Crawford and Crawford 1967).

The exact role of thrombosis in C.H.D. is also confused. For many years there was general acceptance that acute heart attacks were usually the result of thrombosis occurring in one or more atherosclerotic arteries. Since then, however, evidence has accumulated that a proportion of patients who die suddenly have no demonstrable coronary thrombosis, although they may have severe coronary artery atherosclerosis (Spain et al 1969; Friedman et al 1973).

Not only are the relationships between a) C.H.D. and atherosclerosis, and b) C.H.D. and thrombosis confused, but the exact role of thrombosis in the development of atherosclerosis also has a long history of debate. Although the precise mode of initiation of atherosclerosis is not known, it seems likely that thrombosis may not play the central role at this stage of plaque formation. However, there is evidence that thrombosis is the major factor determining extension of atheromatous lesions and thrombosis certainly is important in the pathogenesis of clinically evident occlusive C.H.D.

Perhaps in view of the evidence implicating thrombosis in the evolution of atherosclerosis, there has been a reawakening of interest in the role of the haemostatic system in the pathogenesis of cardiovascular disease. There has been a veritable explosion of interest in the possible role of platelets, not only in thrombosis but also in atherogenesis.

Considerable interest has also been shown in the postulate that normally in vivo coagulation and fibrinolytic activity are in equilibrium and that imbalance in favour of fibrin deposition will lead to an increased thrombotic tendency and accelerate atherosclerotic changes.

Prospective epidemiological studies are currently underway investigating in detail various parameters of the haemostatic system in relation to eventual C.H.D. (Meade and North 1977). Much of the work already reported has been concerned with identifying factors likely to promote increased fibrin formation but the haemostatic equilibrium may be upset if there is a reduced capacity to lyse even normal amounts of fibrin. Thus, it is postulated, defective fibrinolytic activity may increase the tendency to form stable thrombi.

Because of the relative lack of information about fibrinolytic activity in patients with arterial disease and in particular with C.H.D., it seemed worthwhile attempting to establish whether or not the levels of plasma fibrinolytic activity in patients with C.H.D. were

the same or different from the levels recorded in 'normal' subjects.

The aetiology of C.H.D. is multifactorial many different so-called "risk factors" having been investigated and many rejected. It is clear however that certain groups of individuals are at increased risk of developing clinically evident C.H.D. In this thesis the levels of plasma fibrinolytic activity have been examined in sub-groups of individuals with evidence of accepted C.H.D. "risk factors" and compared with the levels found in individuals considered to be at less risk.

REFERENCES.

CRAWFORD, T. & CRAWFORD, M.D. (1967). Prevalence and pathological changes of ischaemic heart disease in a hard-water and in a soft-water area. Lancet, 1, 229.

FRIEDMAN, M., MANWARING, J.H., ROSENMAN, R.H., DONLON, G., ORTEGA, P., & GRUBE, S.M. (1973). Instantaneous and sudden deaths. Clinical and pathological differentiation in coronary artery disease. Journal of the American Medical Association, 225, 1319.

FULTON, M., ADAMS, W., LUTZ, W., & OLIVER, M.F. (1978). Regional variations in mortality from ischaemic heart and cerebrovascular disease in Britain. British Heart Journal, 40, 563.

Joint Working Party report of the Royal College of Physicians of London and the British Cardiac Society (1976). Prevention of coronary heart disease. Journal of the Royal College of Physicians, Vol.10, No.3.

MEADE, T.W., & NORTH, W.R.S. (1977). Population based distributions of haemostatic variables. British Medical Bulletin, 33, 283.

MORRIS, J.N., & CRAWFORD, M.D. (1958). Coronary heart disease and physical activity of work. Evidence of a national necropsy study. British Medical Journal, 2, 1485.

SPAIN, D.M., BARDESS, V.A., MATERO, A., & TARTER, R. (1969). Sudden death due to coronary atherosclerotic heart disease. Journal of the American Medical Association. 207, 1347.

World Health Statistics Annual 1972 (1975). World Health Organization, Geneva.

CHAPTER I.

ATHEROSCLEROSIS AND THROMBOSIS.

INTRODUCTION.

Before embarking upon the main substance of this thesis, it would seem useful to summarise some current concepts of the nature of atherosclerosis and atherogenesis and to review the sequence of structural events in arterial thrombosis.

ATHEROSCLEROSIS-MORPHOLOGICAL FEATURES.

Atherosclerosis is often a frustrating disease to study. It is a lifelong disease which has no symptoms in its early stages and since there is, as yet, no adequate non-invasive method of detecting its presence, it is often diagnosed only when the end-organs supplied by the vessels are severely damaged. The problem is further complicated by the fact that there is a lack of suitable experimental animal models: man appears to be unique in suffering from spontaneous occlusive atherosclerotic disease.

Crawford (1961) has succinctly defined atherosclerosis as "a common process which affects the

large elastic and muscular arteries and which is characterised by focal intimal thickening". The thickened areas consist of proliferated connective tissues, tissue debris and accumulations of plasma-derived lipids and other plasma constituents. The process is at once both degenerative and proliferative - these two characteristics being epitomized by the accumulation of lipid and intimal cell necrosis on one hand and, on the other, by brisk proliferation of smooth muscle cells.

Normal Intima.

In very young children there is virtually no intima, the endothelium being separated from the internal elastic lamina only by small amounts of glycosaminoglycan and collagen fibres. After the first decade there is a gradual increase in subendothelial connective tissue. Low density lipoprotein (LDL) and most other plasma macromolecules are present in normal intima. In young children LDL is present in small amounts only but the levels increase in the second decade and reach adult levels by the late twenties (Smith 1974).

The Fatty Streak.

From early infancy onwards, tiny deposits of lipids may be visible in the intimae of large arteries such as the aorta. They have been recorded even in foetal arteries (Sinzinger et al 1975). In the aorta and in the

coronary arteries, these lesions initially appear as discrete, barely elevated, yellowish dots. Eventually these dots coalesce to form the yellow streak lesions which are commonly visible naked-eye at autopsy. It is not known whether or not these fatty streaks are related to the classical fibrolipid plaques archetypal of atherosclerosis since the common and ubiquitous juvenile fatty streak is a poor predictor for clinically significant disease (McGill, 1968).

In the common type of juvenile fatty streak the lipid is intracellular and there is little connective tissue proliferation. However another type of fatty streak, occurring in young adults, has been described (McGill 1974) in which the lipid is extra-cellular and there is necrosis of intimal smooth muscle cells. It is suggested that these lesions may be progressive and consistute precursors of fibrolipid plaques.

Gelatinous Lesions.

These tiny translucent, colourless or pale pink lesions are easily missed unless careful examination of the aortic intima is made. There is very little data relating to their frequency or distribution (Smith 1975). Histologically they consist of oedematous separation of thickened collagen fibres and they contain increased amounts of all plasma consistuents but especially fibrinogen and low density lipoprotein (Smith 1975).

In coronary arteries they have been found

microscopically, starting in the second decade (Velican and Velican 1979).

The place of these lesions in the atherosclerotic order is poorly understood.

The Fibrolipid Plague.

This is regarded as the archetypal lesion of atherosclerosis and its frequency in different populations is a good predictor of occlusive arterial disease. These lesions consist essentially of a yellow, lipid-rich, basal pool covered by a connective tissue cap of variable thickness. In some the cap is the predominant element and the lesion has a pearly white appearance but in others the basal pool of lipid and tissue debris may be massive and separated from the vessel lumen by only a thin cap of fibromuscular tissue. Other morphological features such as calcification, haemorrhage, ulceration and thrombosis are frequently superimposed on this basic morphology. In the mass of lipid under the cap there is marked increase in the proportion of free (unesterified) cholesterol but the major component is cholesterol ester probably derived from plasma LDL although the LDL content of the actual lesion is low (Smith 1981).

Fibrin in Arterial Wall Lesions.

Smith et al (1976), have described a method for assessing the concentration of fibrin present in normal

intima and in lesions by removing intimal fibrinogen and then digesting the insoluble fibrin to D and E fragments which can be measured. Normally, in healthy intima, fibrinogen and fibrin are present in the same low concentrations. Early gelatinous lesions show a relatively greater increased content of lipoprotein and fibrinogen than fibrin. In lipid-rich areas of gelatinous plaques fibrin is the major plaque component but there are also high levels of fibrinogen, free lipoprotein and bound lipoprotein. High concentrations of fibrin are associated with high levels of bound lipoprotein which can be released by plasmin - suggesting that the lipoprotein is bound to fibrin possibly at the collagen fibrin interface (Smith 1981).

ATHEROGENESIS.

Since smooth muscle cell proliferation seems to be an integral part in the formation of atherosclerotic plaques, insight into the causes of arterial smooth muscle cell proliferation is essential in the understanding of plaque formation. Without the proliferation of modified smooth muscle cells in the arterial intima, it is unlikely that the typical atherosclerotic plaque could develop, for, not only do these cells constitute the most significant element of the plaque cell population, but they also synthesize the extracellular connective tissue matrix which is such an important part of the plaque substance (McCullagh and Balian 1975, Narayanan et al 1976).

Plaque Initiation.

Two main theories of atherogenesis currently vie for support. The first is an old hypothesis which states plaques form in response to injury to the arterial wall. The second, competing, hypothesis was first proposed about five years ago and states that the plaques are benign tumours.

Response to Injury Theory:

Since atherosclerosis is an intimal lesion, the endothelium and factors which injure it are important. Blood constituents gain access to the vessel wall where the endothelium is injured and the permeability of the wall is increased. Plasma proteins accumulate at sites of vessel injury (Mustard et al 1974) and cholesterol from the blood also preferentially accumulates where there is increased protein accumulation (Somer and Schwartz 1972). It has been shown that, both in experimental animals and in humans, early vessel wall atherosclerotic changes occur at sites of vessel injury (Jorgensen et al 1972, Moore 1973).

A direct link between vessel injury, platelets and atherosclerosis was established when Ross and his colleagues (1974) observed that when blood clots, the platelets release a factor which stimulates smooth muscle proliferation. They have shown that aggregated platelets release a heat-stable, low molecular weight (MW 13,000

daltons), basic protein which stimulates smooth muscle cells from monkey arteries to synthesize DNA and divide so that the cell population in cultures doubles within 48 - 60 hours of adding this platelet growth stimulating protein.

Endothelial damage to vessels in a variety of animal models has been shown to lead to the rapid migration of smooth muscle cells from the media into the intima where they proliferate (Ross and Glomset 1976, Stemerman and Spaet 1972.) With a single injury the resulting lesions do not appear to contain much lipid with the smooth muscle but repeated injury produces raised atheromatous lesions rich in cholesterol (Friedman et al 1975).

Further evidence of the importance of platelets in the initiation of intimal smooth muscle cell proliferation has been obtained from studies of animals made thrombocytopenic (Moore et al 1976)and in animals given drugs to inhibit platelet interaction with the vessel wall (Harker et al 1976). In all of these, vessel injury resulted in less atherosclerosis than in normal animals similarly injured.

Proponents of the response to injury hypothesis assert that, in animal models, almost any type of chronic injury, mechanical, chemical or immunological leads to the development of lesions resembling human atherosclerotic plaques. Supporters of this hypothesis suggest that humans may suffer arterial injury from hypertension, from antibodies to, or carbon monoxide from cigarette smoke and from high concentrations of blood lipids.

The Monoclonal Hypothesis of Smooth Muscle Cell Proliferation:

In 1973 Benditt and Benditt proposed a new hypothesis, the monoclonal theory of atherogenesis which proposed the smooth muscle cell proliferation within the fibrous plaques was monoclonal in type. Thus the process is more closely related to a benign smooth muscle tumour than to repair following injury.

This approach stems originally from observations made by Lyon (1961) relating to the random inactivation of one X chromosome in every adult female cell. Usually this is of no significance, since both X chromosomes code for similar enzymes.

However, glucose - 6 - phosphate dehydrogenase (G6PD) is a special case since it can exist in at least two electrophoretically different isoenzyme forms - type A and type B. The G6PD gene therefore acts as a "marker" gene.

Analysis of atheromatous plaques from Negro females heterozygous for G6PD showed that the plaques consisted solely or at least predominantly of cells containing only one isoenzyme, A or B, whereas in the surrounding lesion-free aortic intima both isoenzymes A and B were present. (Pearson et al 1975, 1977, Benditt 1977). The conclusion was that the plaques must have arisen from a single type of cell - hence the term "monoclonal". The recognised "risk factors" for example, cigarette smoking, low density and very low density lipoproteins are compatible with this theory since they may act as the mutagens which trigger the proliferative process.

Not all workers, however, accept Benditts interpretation of his findings (Martin et al 1974), on the grounds that there may be other explanations of the finding of only one cell type in each lesion.

Plague Growth - The Role of Thrombosis.

The "Thrombogenic Theory":

The suggestion that thrombosis may play a part in the growth of atherosclerotic plaques is by no means new, since it was first presented some 135 years ago. The "thromogenic or encrustation theory" was formulated originally by von Rokitansky in his Handbook of Pathological Anatomy which appeared first in German in 1842 and was translated into English ten years later. Von Rokitansky believed that substances derived from the blood, in particular fibrin, were deposited on the endothelial surface of the arteries. These substances, he considered, probably underwent fatty metamorphosis and other degenerative changes with the eventual formation of a pulpy mass containing cholesterol crystals, fat globules, albumin and calcareous salts. Von Rokitansky thus described fairly typical atherosclerosis. As quoted by Duguid (1976), in 1936, almost a hundred years after Von Rokitansky's original publication, Clark et al further emphasised the role of thrombosis in atherogenesis. They examined atherosclerotic plaques and grossly identifiable mural thrombi on the intimal surface of human aortae and coronary arteries and were impressed by the gradual transition of mural thrombi, to deposits

which were covered by endothelium and which were partly or entirely organised to collagenous tissues containing proliferating cells. Other plaques showed evidence of multiple layers of homogenous material with the staining properties of fibrin separated from each other by fibrous tissue. This they interpreted as recurrent deposition of blood elements with subsequent organisation.

However, Clark's work seems to have had very little impact on concepts prevalent at the time about the nature of the disease and perhaps the name most commonly associated with the "thrombogenic theory" is that of Duguid. In a monograph published in 1976 Duguid relates how shortly after the Second World War, he reviewed the "thrombogenic theory" and extended it to explain a number of aspects of atherosclerosis (Duguid 1946, 1948). In a series of publications he stressed two features fundamental to an understanding of the "thromogenic hypothesis". Firstly, he pointed out that if the increased fibrous tissue demonstrable in the arterial intima in atherosclerosis was the result of preceding degenerative changes then it would tend to reduce vascular elasticity and cause dilatation of the arteries, and not produce the narrowing characteristically observed in atheromatous vessels. When a mural thrombus persists on an arterial surface it becomes covered by endothelium proliferating from the adjoining unaffected surface and it becomes incorporated into the intima by organisation causing thickening of the vessel wall. Recurrent thrombotic episodes result in significant reduction of the

arterial lumen. Secondly, Duguid stressed that the processes of incorporation into and merging with the intima would tend to obscure the thrombotic origin of the lesion.

Identification of Thrombotic Residua:

Duguid's morphological observations were soon confirmed, (Heard 1949, Crawford and Levene 1952) but until about 20 years ago both the supporters and the opponents of the "thrombogenic theory" tended to refer to fibrin deposition rather than mural thrombosis, as if the two terms were synonymous.

However, it must be realised that the finding of fibrin on or within the arterial intima does not itself constitute a prima facie case that mural thrombosis has occurred since there may be other explanations for its presence. It is of interest that the important role of platelets in the formation of thrombi has had to be re-established in connection with the thrombogenic theory of atherosclerosis. The finding of platelet aggregates within plaques provides much stronger evidence of mural thrombi. Using a fluorescent antibody technique Carstairs (1965) described the presence of platelets in atherosclerotic lesions. Subsequently it has been shown that platelet antigens exist only in fibrolipid plaques and not in fatty streaks (Woolf and Carstairs 1967, Hudson and McCaughey 1974).

Residua of mural thrombi may be seen in up to 90% of lesions in any single aorta and in the coronary arteries

in up to 33%. The frequency with which such incorporated thrombi occur correlates well with the incidence of coronary arterial occlusion and it has been shown that smoking and hypertension may have an incremental effect on the incidence of thrombotic residua. (Woolf et al 1969).

Experimental Models:

Many animal models have been devised to study the natural history of mural thrombi (Woolf et al 1968, Moore 1973). Within a few days of thrombus induction endothelialization can be seen (Davies et al 1975) and about 3 weeks later a fibromuscular plaque containing quantities of lipid are observed. These lesions closely resemble the fibrolipid found in human arteries.

The Role of Thrombosis:

It appears, therefore, that mural thrombi occur frequently in association with human atherosclerotic lesions and from the animal experiments it seems that the presence of a thrombus can elicit a brisk proliferative response in the subjacent wall. Although the "thrombogenic theory" has a definite place in any consideration of the evolution of atherosclerosis, there is certainly not universal agreement on the precise role of thrombosis in atherosclerosis. Not all features of atherosclerosis can be explained on the basis of the "thrombogenic theory", in particular, the theory as proposed does not explain why thrombi are deposited in the first place. From current evidence, it seems most likely

that the major role of thrombosis is in plaque growth and in the progression of atherosclerotic lesions. Thrombosis is less likely to be a significant factor in the initiation of the disease, even though embolic thrombi have been shown to be able to give rise to plaques in previously normal arteries (Chandler and Terrell Pope 1975).

STRUCTURAL ASPECTS OF ARTERIAL THROMBOSIS.

The fate of a thrombus depends on many different factors including its composition, its site and its stability. Perhaps one of the more important and often-forgotten features of thrombosis is that the thrombi themselves are in a continuous state of formation and dissolution.

Mechanisms in the Formation and Dissolution of Thrombi.

Early investigators realised that thrombus formation involved a complex series of interactions between the vessel wall and blood components, both cellular and plasmatic. Our understanding of the processes involved has expanded rapidly over the past 20 years but there are still many gaps in our knowledge.

When the endothelial surface of a vessel is damaged, platelets come into contact with subendothelial structures, and a series of reactions which result in the formation of a platelet aggregate and subsequently a

thrombus is initiated. Platelets adhere to certain subendothelial structures including the basement membrane, collagen and microfibrils. (Hovig 1963, Stemerman et al 1971, Baumgartner 1974). When platelets adhere to collagen they are stimulated to release the contents of their amine storage granules.

In addition to releasing their granule content, when platelets come into contact with collagen, free arachidonate is formed from membrane phospholipids. This arachidonate is converted enzymatically to a number of substances including the prostaglandin endoperoxides - PGG_2 and PGH_2 - and thromboxane A_2 (Hamberg et al 1975, Bills et al 1976). Both the ADP released from the amine storage granules and the arachidonate intermediates can cause disc shaped platelets to change shape and become spheres with pseudopods. (Kinlough-Rathbone et al 1976). Platelets which have undergone this shape change are able to stick to each other and to platelets adherent to the vessel wall, resulting in the formation of platelet aggregates.

Endothelial damage and the platelet reaction also result in activation of the coagulation system. Factor XII is activated by collagen (Niewiarowski et al 1966), and when the endothelium is injured, tissue thromboplastin becomes available (Nemerson and Pitlick 1972). When platelets comes into contact with each other Factor XII is activated. (Walsh 1972a). In addition, platelets may also directly activate Factor XI when they come into contact with collagen. (Walsh 1972b). The coagulation reactions

are further accelerated by the presence of platelet membrane phospholipids (Joist et al 1974), so that in and around a platelet aggregate, thrombin is generated. Thrombin itself causes platelets to change shape and adhere to each other, to release their granule contents (including ADP) and to produce prostaglandin endoperoxides and thromboxane A_2. The ADP and arachidonate products induce further platelet adhesion so that the platelet mass grows.

Stabilisation of the platelet aggregate results when sufficient thrombin is formed to convert fibrinogen to fibrin and the polymerzing fibrin adheres tightly to the platelet mass (Niewiaroski et al 1972). The fibrin which forms around the platelet mass holds the platelets together (Hirsh et al 1968). Without it, platelet aggregates break up readily under the influence of flowing blood.

Vessel wall damage causes the release of plasminogen activators (Nilsson and Pandolfi 1970) probably from the endothelial cells. Further activation of plasminogen to plasmin results from the activation of Hageman factor. Since the platelet aggregates break up readily, lysis of the fibrin holding the platelet mass together can disrupt the thrombus. Thus fibrinolytic activity may be an important factor in deciding the eventual fate of the platelet aggregate - dissolution or persistence as a thrombus.

Patterns of blood flow are important in determining the size of a thrombus. Thrombus formation is greater in

regions of disturbed blood flow (Goldsmith 1972), where particles of platelets become trapped in vortices long enough to facilitate their interactions with each other, and with the vessel wall. Clinically occlusive arterial thrombi seldom occur except in areas of disturbed blood flow, such as at a stenosis. In areas where there is a high flow rate, the shear caused may dislodge part or all of a thrombus.

It is of clinical importance that thrombi are essentially dynamic structures. A mural thrombus may form at a site of vessel damage, persist for a brief period then embolize into the distal circulation. Reformation of a thrombus at the injured site usually occurs but this seems to be normally a limited process and it is not known how long an injured vessel wall can stimulate platelets to interact with it. In fact, the available evidence suggests that the initial stimulating effect of the subendothelial structures may be lost rapidly in vivo.

The Effect of Thrombus Formation on the Vessel Wall.

Thrombi affect vessel walls where they come into contact with them, producing further damage. On activation, platelets release a number of substances including arachidonate intermediates, nucleotides, serotonin and cationic protein, which increase vessel wall permeability (Packham et al 1968, Nachman et al 1972). Activated platelets also release or form collagenase and elastase which can digest collagen and elastin in the

vessel wall. (Chesney et al 1974, Robert et al 1971). When platelets are stimulated they form a material known as H.E.T.E. (12 - L - hydroxy - 5,8,10,14 - eicosatetraenoic acid) which is chemotactic for polymorphonuclear leukocytes (Turner et al 1975) and a factor which interacts with the fifth component of complement to produce a chemotactic material (Weksler and Coupal 1973). The polymorphs attracted by these agents to the injury site may also release substances which affect the vessel wall.

CONCLUSION.

It is abundantly clear that the process involved in the inititation of atherosclerosis and the evolution of plaques to cause clinically significant disease involve a highly complex and interactive series of events. This being so it is evident from the outset that the aetiology and pathogenesis of atherosclerotic disease must be truly multifactorial.

The development and subsequent persistence of clinically significant thrombi may be the result of imbalance between fibrin formation and dissolution. Thrombolysis may be impaired in subjects with reduced fibrinolytic activity.

It is the aim of this thesis not to become involved in the debate about the mode of initiation of atherosclerosis but rather, accepting that thrombosis is important in the evolution of lesions, to examine the

hypothesis that patients with atherosclerosis, in particular coronary heart disease, or at increased risk of developing coronary atherosclerosis may include a population whose thrombotic tendency is increased by poor fibrinolytic activity.

REFERENCES.

BAUMGARTNER, H.R. (1974). The subendothelial surface and thrombosis. Thrombosis et Diathesis Haemorrhagica, Supplement 59, 91.

BENDITT, E.P. & BENDITT, J.M. (1973). Evidence for a monoclonal origin of human atherosclerotic plaques. Proceedings of the National Academy of Sciences of the United States of America. 70, 1753.

BENDITT, E.P. (1977). Implications of the monoclonal character of human atherosclerotic plaques. American Journal of Pathology, 86, 693.

BILLS, T.K., SMITH, J.B. & SILVER, M.J. (1976). Metabolism of (^{14}C) arachidonic acid by human platelets. Biochimica Biophysica Acta, 424, 303.

CARSTAIRS, K.C. (1965). The identification of platelets and platelet analysis in histological sections. Journal of Pathology and Bacteriology 90, 225.

CHANDLER, A.B.& TERRELL POPE, J., (1975) In Blood and Arterial Wall in Atherogenesis and Arterial Thrombosis. I.F.M.A. Scientific Symposium No.4 Ed. by Hautvast, J.G.A.J., Hermius, R.J.J., Van der Haap, F. p.111. E.W. Leiden, E.J. Brill.

CHESNEY, C. McI., HARPER, E.& COLMAN, R.W. (1974). Human platelet collagenase. Journal of Clinical Investigation, 53, 1647.

CLARK, E., GRAEF, I, & CHASIS, H. (1936). Thrombosis of the aorta and coronary arteries with special reference to the 'fibrinoid' lesions. Archives of Pathology, 22, 183.

CRAWFORD, T., & LEVENE, C.I. (1952). The incorporation of fibrin in the aortic intima. Journal of Pathology and Bacteriology, 64, 523.

CRAWFORD, T.(1961). Morphological aspects in the pathogenesis of atherosclerosis. Journal of Atherosclerosis Research 1, 3.

DAVIES, M.J., BALLANTINE, S.J., ROBERTSON, W.B.,& WOOLF, N.(1975). The ultrastructure of organising experimental mural thrombi in the pig aorta. Journal of Pathology, 117, 75.

DUGUID, J.B., (1946). Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. Journal of Pathology and Bacteriology, 58, 207.

DUGUID, J.B., (1948). Thrombosis as a factor in the pathogenesis of aortic atherosclerosis. Journal of Pathology and Bacteriology 60, 57.

DUGUID, J.B. (1976). The dynamics of atherosclerosis, Aberdeen University Press.

FRIEDMAN, R.J., MOORE, S. & SINGAL, D.P. (1975). Repeated endothelial injury and induction of atherosclerosis in normolipemic rabbits by human serum. Laboratory Investigation, 30, 404.

GOLDSMITH, H.L. (1972). The flow of model particles and blood cells and its relation to thromogenesis. In "Progress in Hemostasis and Thrombosis". (Ed. by Spaet, T.H.Vol.1, p.97, Grune and Stratton, New York and Academic Press, London.

HAMBERG, B., SVENSSON, J. & SAMUELSSON, N. (1975). Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proceedings of the National Academy of Sciences, U.S.A., 72, 2994.

HARKER, L.A., ROSS, R., SLICHTER, S.J. & SCOTT, C.R. (1976). Homocystine-induced arteriosclerosis. The role of endothelial cell injury and platelet response in its genesis. Journal of Clinical Investigation, 58, 731.

HEARD, B.E., (1949). Mural thrombosis in renal artery and its relation to atherosclerosis, Journal of Pathology and Bacteriology. 61, 635.

HIRSCH, J., BUCHANAN, M., GLYNN, M.F.& MUSTARD, J.R. (1968). Effect of streptokinase on hemostasis. Blood, 32, 726.

HOVIG, T. (1963). Release of a platelet aggregating substance (adenosine diphosphate) from rabbit blood platelets induced by saline "extract" of tendons. Thrombosis et Diathesis Haemorrhagica, 9, 264.

HUDSON, J., & McCAUGHEY, W.T.E. (1974). Mural thrombosis and atherogenesis in coronary artery and aorta. An investigation using antifibrin and antiplatelet sera. Atherosclerosis, 19, 543.

JOIST, J.H., DOLEZEL, G., LLOYD, J.V., KINLOUGH-RATHBONE, R.L. & MUSTARD, J.F. (1974). Platelet factor-3 availability and the platelet release reaction. Journal of Laboratory and Clinical Medicine, 84, 474.

JORGENSEN, L., PACKHAM, M.A., ROWSELL, H.C., & MUSTARD, J.F. (1972). Deposition of formed elements of blood in the intima and signs of intimal injury in the aorta of rabbit, pig and man. Laboratory Investigation, 27, 341.

KINLOUGH-RATHBONE, R.L., REIMERS, H.-J., MUSTARD, J.F., & PACKHAM, M.A. (1976). Sodium arachidonate can induce platelet shape change and aggregation which are independent of the release reaction. Science, 192, 1011.

LYON, M.F., (1961.) Gene action in the X chromosome of the mouse. Nature 190, 372.

McCULLAGH, K.A., & BALLIAN, G. (1975). Collagen characterisation and cell transformation in human atherosclerosis. Nature, 258, 73.

McGILL, H.C. Jr. (1974). In Atherosclerosis Vol.3 Ed. by Schettler, G., Weizel, A., p27. Springer-Verlag, Berlin, Heidleberg, New York,

McGILL, H.C., (1968). Fatty streaks in the coronary arteries and aorta. Laboratory Investigation 18, 560.

MARTIN, G.M., SPRAGUE, C.A., NORWOOD, T.H. & PENDERGRASS W.R. (1974). Clonal selection attenuation and differentiation in an in vitro model of hyperplasia. American Journal of Pathology, 74, 137.

MOORE, S. (1973). Thromboatherosclerosis in normolipaemic rabbits: a result of continued endothelial damage. Laboratory Investigation, 29, 478.

MOORE, S., FRIEDMAN, R.J., SINGAL, D.P., GAULDIE, J., BLAJCHMAN, M.A., & ROBERTS, R.S. (1976). Inhibition of injury induced thromboatherosclerotic lesions by antiplatelet serum in rabbits. Thrombosis and Haemostasis, 35, 70.

MUSTARD, J.F., PACKHAM, M.A., MOORE, S., & KINLOUGH-RATHBONE, R.L. (1974). Thrombosis and atherosclerosis. In "Atherosclerosis" Ed. by Schettler G. & Weizel, A. Vol.III, p.253, New York, Springer.

NACHMAN, R.L., WEKSLER, B., & FERRIS, B. (1972). Characterization of human platelet vascular permeability-enhancing activity. Journal of Clinical Investigation, 51, 549.

NARAYANAN, A.S., SANDBERG, L.B., ROSS, R., & LAYMAN, D.L. (1976). The smooth muscle cell: III: Elastin synthesis in arterial smooth muscle cell culture. Journal of Cell Biology, 68, 411.

NEMERSON, Y., & PITLICK, F.A. (1972). The tissue factor pathway of blood coagulation. In "Progress in Haemostasis and Thombosis" Ed. by Spaet, T.H., Vol.1, p.1, New York, Grune and Stratton, London Academic Press.

NIEWIAROWSKI, S., STUART, R.K., & THOMAS, D.P. (1966). Activation of intravascular coagulation by collagen. Proceedings of the Society of Experimental Biology, 123, 196.

NIEWIAROWSKI, S., REGOECZI, E., STEWART, G.J., SENYI, A., & MUSTARD, J.F. (1972). Platelet interaction with polymerizing fibrin. Journal of Clinical Investigation, 51, 685.

NILSSON, I.M., & PANDOLFI, M. (1970). Fibrinolytic response of the vascular wall. Thrombosis et Diathesis Haemorrhagica, Supplement 40, 231.

PACKHAM, M.A., NISHIZAWA, E.E., & MUSTARD, J.F. (1968). Response of platelets to tissue injury. Biochemistry and Pharmacology Supplement 171.

PEARSON, T.A., WANG, A., SOLEZ, K., & HEPINSTALL, R.H. (1975). Clonal characteristics of fibrous plaques and fatty streaks from human aortas. American Journal of Pathology, 81, 379.

PEARSON, T.A., KRAMER, E.C., SOLEZ, K., & HEPINSTALL, R.H. (1977). The human atherosclerotic plaque. American Journal of Pathology, 86, 657.

ROBERT, B., ROBERT, L., LEGRAND, Y., PIGNAUD, G., & CAEN, J. (1971). Elastolytic protease in blood platelets. Series Haematologica, 4, 75.

ROSS, R., & GLOMSET, J.A. (1976). The pathogenesis of atherosclerosis. New England Journal of Medicine, 295, 369.

ROSS, R., GLOMSET, J., KARIYA, B., & HARKER, L. (1974). A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proceedings of the National Academy of Sciences, U.S.A., 71, 1207.

SINZINGER, H., FEIGL, W., DADAK, C., & HOLZNER, J.H. (1975). Intimal alterations of the aorta and the great arteries of newborn and children. Pathologia et Microbiologia, 42, 129.

SMITH, E.B., (1974). The relationship between plasma and tissue lipids in human atherosclerosis. Advances in Lipid Research 12:1.

SMITH, E.B., (1975). The Smooth muscle of the artery. In Advances in Experimental Medicine and Biology, Vol.57. Ed. by Wolf S. & Werthesson, N.T. Vol.57, p.254. New York, London: Plenum Press.

SMITH, E.B., ALEXANDER, K.M., & MASSIE, I.B. (1976). Insoluble fibrin in human aortic intima. Quantitative studies on the relationship between insoluble fibrin, soluble fibrinogen and LD lipoprotein. Atherosclerosis, 23: 19.

SMITH, E.B. (1981). Relationship between lipids and atherosclerosis. In. Haemostasis and Thrombosis Ed. by Bloom, A.L.& Thomas D.P. p554, Edinburgh, Churchill Livingstone.

SOMER, J.B., & SCHWARTZ, C.J. (1972). Focal (^{3}H) cholesterol uptake in the pig aorta. Part 2. Distribution of (^{3}H) cholesterol across the aortic wall in areas of high and low uptake in vivo. Atherosclerosis, 16, 377.

STEMERMAN, M.B., BAUMGARTNER, H.R., & SPAET, T.H. (1971). The subendothelial microfibril and platelet adhesion. Laboratory Investigation, 24, 179.

STEMERMAN, M.B., & SPAET, T.H. (1972). The subendothelium and thrombogenesis. Bulletin of New York Academy of Science, 48, 289.

TURNER, S.R., TAINER, J.A., & LYNN, W.S. (1975). Biogenesis of chemotactic molecules by the arachidonate lipoxygenase system of platelets. Nature, London, 257, 680.

VELICAN D., & VELICAN, C., (1979). Study of fibrous plaques occurring in the coronary arteries of children. Atherosclerosis 33, 201.

VON ROKITANSKY, C. (1844). In Handbuch der Pathologischen Anatomie, Vo.2. Braunsmuller and Seidel, Vienna, p.306. Translated into English by Day 1852 in a Manual of Pathological Anatomy 4, 261. London Sydenham Society.

WALSH, P.N. (1972a). The role of platelets in the contact phase of blood coagulation. British Journal of Haematology, 22, 237.

WALSH, P.N. (1972b). The effects of collagen and kaolin on the intrinsic coagulant activity of platelets. Evidence for an alternative pathway in intrinsic coagulation not requiring factor XII. British Journal of Haematology, 22, 393.

WEKSLER, B.B., & COUPAL, C.E. (1973). Platelet-dependent generation of chemotactic activity in serum. Journal of Experimental Medicine, 137, 1419.

WOOLF, N., & CARSTAIRS, K.C. (1967). Infiltration and thrombosis in atherogenesis. A study using immunofluorescent techniques. American Journal of Pathology, 51, 373.

WOOLF, N., SACKS, M.I., & DAVIES, M.J. (1969). Aortic plaque morphology in relation to coronary artery disease. American Journal of Pathology, 57, 187.

WOOLF, N., BRADLEY, J.W.P., CRAWFORD, T., & CARSTAIRS, K.C. (1968). Experimental mural thrombi in the pig aorta. The early natural history. British Journal of Experimental Pathology, 49, 257.

CHAPTER II.

"RISK FACTORS" AND CORONARY HEART DISEASE.

INTRODUCTION.

Much energy has been expended in the attempt to identify individuals or groups of individuals at particular risk of coronary heart disease (C.H.D). The term "risk factor" is widely used to describe characteristics found in groups of healthy individuals which are related to a subsequent increased incidence of C.H.D. It must be emphasised that "risk factors" are associated only with an increased future risk of disease and the term is not generally meant to imply that there is always established proof of a causal relationship.

In spite of the vast amounts of research effort which have been poured into determining the so-called "risk factors" for C.H.D., these factors have proved at best only group markers. Prospective studies, such as the Framingham Study, certainly show that "high risk" patients (as defined in terms of three major "risk factors" - hypertension, raised lipid levels and cigarette smoking) are about three times more likely to develop C.H.D. than patients in the "low risk" category, but these comparisons are between groups at risk - "high risk" group compared

with "low-risk" group. Within a "high-risk" group these markers are of poor predictive value. In a group of 100 men with the three major "risk factors" only eight will have clinical signs of C.H.D. in the subsequent 10 years and ninety-two will not (Intersociety Commission for Heart Disease Resources 1970).

Certain "risk factors" identify an individual with "high risk status" but are not modifiable. Of these non-modifiable "risk factors", age and sex are obviously the two most important. Other "risk factors" such as smoking and blood lipid levels are, however, potentially modifiable and it is these factors which have provoked particular interest in terms of possible C.H.D. prevention.

In a multivariate study reported by Keys et al (1972), five variables were included: age, systolic blood pressure, serum cholesterol, smoking and body mass index. They did not find that body mass index (defined as a function, weight/height^2) contributed significantly to the overall risk in the presence of other variables in either the European populations or the American populations studied. It would appear that the three most important modifiable "risk factors" are hypertension, blood lipid levels and cigarette smoking.

It is the purpose of this chapter to consider each of the major C.H.D. "risk factors" in turn and to examine briefly the importance and, where possible, the likely mechanism of the risk associated with each of these factors.

AGE AND SEX.

It is certainly popularly believed that men in their forties and fifties are the section of the community most commonly afflicted by C.H.D. and indeed, in the United Kingdom, hospital admissions for C.H.D. do rise to a peak in middle-aged men. However, the age-specific incidence and mortality rates for C.H.D. rise more or less logarithmically throughout life, approximately doubling for every ten years of advancing age. The male: female ratio diminishes progressively from a maximum of around 6 : 1 in the under 40 year olds to near equality around 80 years. (Joint Working Party Report 1976).

HYPERTENSION.

Hypertension is recognised as one of the major precursors of atherosclerotic vascular lesions. The clinical evidence is compelling. In coarctation of the aorta, atherosclerotic changes are found predominantly in the high pressure area above the constriction and in rheumatic mitral valve disease the resulting pulmonary hypertension is associated with pulmonary artery atherosclerosis.

The epidemiological evidence from prospective studies is equally convincing. In the Framingham population, Kannel and Dawber (1974), demonstrated that a significant independent relationship exists between the height of the

blood pressure, particularly at systole, and the probability of developing C.H.D. Essential hypertension is presumed to exist when the blood pressure rises above some arbitrary value (most commonly 160/95 mmHg). Certainly this level serves to identify individuals at high risk from all the major cardiovascular diseases but the risk is related to the height of the blood pressure, even in non-hypertensives, and there is no discernible threshold. For men in their forties the incidence of C.H.D. increases by almost 20% for each 10 mm. rise in the systolic blood pressure. This means that, with a systolic blood pressure of 160 mmHg, the C.H.D. risk is almost twice that at 110 mmHg. At levels exceeding 180 mmHg the risk gradient becomes even steeper. With increasing age, hypertension becomes more prevalent and its significance as risk factor does not diminish.

Reversal of Risk:

Obviously individuals with severe hypertension are at particular risk but, in community terms, mild hypertension contributes much more to the occurrence of C.H.D. As a general guideline, the Joint Working Party report (1976) suggested that diastolic blood pressure levels of 100 mmHg. or more in men under 65 years should be regarded as justifying therapeutic intervention. Although concrete statistical proof is lacking, there is very suggestive evidence that, amongst middle-aged men, the treatment of hypertension will reduce the risk of C.H.D. (Veterans

Administration Co-operative Study Group 1970) and there is unequivocal evidence from the same study that life is prolonged and morbidity from strokes, congestive failure, aortic aneurysm and renal failure is delayed by controlling hypertension.

Relation to Other "Risk Factors:"

Whilst the risk of C.H.D. is high amongst hypertensives it is not uniformly so, for, although the risk is proportional to the height of the blood pressure, it varies widely depending on associated factors also contributing to it. Coexisting increased lipid levels influence the risk associated with any blood pressure level and to further compound the difficulty, at any given combination of lipid and blood pressure levels, the risk is markedly influenced by other coexisting factors such as cigarette smoking. Risk is further increased if there is electro-cardiographic evidence of left ventricular hypertrophy. Risk can be shown to mount progressively in proportion to the number of abnormalities present in addition to hypertension.

Mechanism of the Risk from Hypertension:

The exact mechanism by which a raised systemic arterial pressure results in atherosclerosis is still a matter of conjecture. It seems atherosclerosis does not

occur at random locations but rather at specific sites of predilection which can be precisely defined and predicted (Texon 1977). A normal or ideal range of blood volume for any given vessel requires a range of pressures and blood flow rates which will not damage the vessel wall. It is likely, that in hypertension, the major factor is local endothelial damage caused by increased shearing stresses and turbulence. However, altered blood flow patterns within an artery also affect certain functional parameters such as the flux of protein across the endothelial barrier (Fry 1973). Fry has also shown that, in experimental models, shearing stresses exceeding 4 mN/cm^2 cause endothelial cells to become deformed and ultimately eroded from the subendothelial connective tissue. Exposure of the subendothelium is followed by the formation of mural thrombi and subsequently by a fibromuscular plaque as described in Chaper I.

A further possible mechanism for the risk associated with hypertension has been suggested by Texon (1977). He postulates that the locally decreased lateral forces, occurring in association with increased blood velocity, act as the initial stimulus producing the intimal proliferation characteristic of atherosclerosis. Once a plaque does develop its presence causes distortion of the blood flow extending further the area of intimal damage.

CIGARETTE-SMOKING.

Expert committees in many countries have concluded that cigarette smoking is a major "risk factor" in coronary heart disease. Evidence from several large studies puts the risk for smokers of dying from C.H.D. at about twice that for non-smokers. (Joint Working Party report 1976). The risk is greatest amongst heavy smokers (smoking more than 20 cigarettes daily) and in those who start smoking at an early age. The relationship with smoking is strong in young people and weakens with age. In a study of male British doctors Doll and Hill (1964) reported that the death rate due to C.H.D. in smokers aged 35 - 44 years was about five times that of non-smokers in the same age range. It has been suggested that the fall in relative risk in smokers with age may be the result of progressive elimination of individuals particularly susceptible to its effect. Some, but not all, studies show that heavy cigarette smoking increases the risk of sudden death.

Reversal of Risk:

Many reports have shown a reduced risk of developing myocardial infarction or of death from C.H.D. in those who stop smoking. Doll and Peto (1976) reported that cessation of cigarette smoking is associated with a reduced risk in normal subjects and Wilhelmsson et al

(1975) reported a similar reduction in risk in patients who stopped smoking after a coronary attack.

The impact of giving up smoking on total mortality is less however than the impact on C.H.D. incidence (Gordon et al 1974). Although a rapid fall of C.H.D. mortality occurs within the first year, it may take ten years or more for the death rate of ex-smokers to reach that of non-smokers. (Ball and Turner 1974).

Relation to Other "Risk Factors:"

The relative risk of smoking is greatly increased by the coexistence of other risk factors such as hypertension or raised plasma cholesterol levels. In terms of cardiovascular disease, the risk is greatest for men, under the age of 65, who have evidence of hypertension, hypercholesterolaemia or diabetes mellitus or who have unexplained ECG abnormalities such as left ventricular hypertrophy.

Mechanism of the Risk of Cigarette Smoking:

The mechanisms involved are not entirely clear. Endothelial damage may occur as a result of increases in heart rate, blood pressure and plasma free fatty acid and triglyceride levels induced by the nicotine stimulated increase in catecholamines (Taylor et al 1974). Frost (1973) exposed rabbits to cigarette smoke and found that they suffered focal losses of endothelial cells with

subseqùent deposition of platelets on the exposed subendothelial tissue. Abnormalities have also been described in the endothelial linings of umbilical arteries of neonates whose mothers smoked (Asmussen and Kjeldsen 1975).

Precursors of mutagens such as aryl hydrocarbons present in cigarette smoke may be important in initiating smooth muscle cell proliferation, a role which would fit into the monoclonal theory of atherogenesis, (Benditt 1977).

The apparently prompt drop in the incidence of C.H.D. when men stop smoking suggests that smoking has some non-cumulative, transient, reversible triggering effect which may be more important than any direct influence on atherogenesis. Inhaled cigarette smoke may increase platelet adhesiveness, raise carboxy haemoglobin levels and increase myocardial irritability. The fact that there is little increased risk associated with cigar and pipe smoking suggests that some particular aspect of cigarette smoking, possibly inhalation, is a key factor.

PLASMA LIPIDS.

An association between lipid metabolism and atherosclerosis has long been inferred. Cholesterol and triglycerides are transported in the plasma bound to proteins in the form of lipoproteins. There are four major families of plasma lipoproteins: the chylomicrons which transport the exogenous or dietary triglyceride; the

very low density lipoproteins (V.L.D.L.) or pre-β lipoproteins, which carry the endogenous triglyceride; the low density lipoproteins (L.D.L.) or β lipoproteins which represent the major transport vehicle for cholesterol; and the high density lipoproteins (HDL) or α lipoproteins, whose main lipid components are phospholipid and cholesterol esters.

Lipid and lipoprotein studies in the past have generally emphasised the positive relationship of total cholesterol, LDL, VLDL and triglyceride to the risk of C.H.D. (Gofman et al 1966; Kannel et al 1971). The higher the concentration of any one of these blood lipids the greater the risk of C.H.D. However, a report on a cohort of the Framingham study population, aged 49 - 82 years (Gordon et al 1977) demonstrated that, in this age range, the major potent lipid "risk factor" was HDL cholesterol which had an inverse association with the incidence of C.H.D. In other words, the lower the HDL level, the greater the risk of C.H.D.

Recognition of the impact of HDL on C.H.D. is not new. In 1951 Barr et al. commented on the reduction of α lipoprotein in atherosclerosis and this was soon confirmed by a report from Finland (Nikkila 1953). In various case control studies, HDL levels have been found to be lower in patients with C.H.D. than in controls. (Oliver and Boyd 1955; Keys and Fidanza 1960). Similarly in prospective studies, Gofman et al (1966) reported lower HDL levels in young men in whom C.H.D. levels subsequently developed.

It seems that, for men at least, HDL is a significant

"risk factor" for C.H.D. even in the younger age ranges. Low levels of HDL are associated with increased risk for C.H.D.

The Framingham study demonstrated that, at least in older men, (aged 49 - 82 years) HDL cholesterol is the major potent lipid "risk factor" and elevated LDL levels are a less significant risk for C.H.D. than are depressed HDL levels. (Gordon et al 1977). LDL cholesterol is however also a "risk factor", there being a positive relationship between LDL levels and C.H.D. LDL is a stronger "risk factor" in men at younger ages than at older ages (Gofman et al 1966).

Raised serum triglyceride concentrations have also been connected with C.H.D. but the evidence remains somewhat ambivalent. In the Framingham study, triglycerides seem to be associated with the incidence of C.H.D. only in women and then only when the levels of other lipids are not taken into account. (Gordon et al 1977). Familial hypertriglyceridaemia seems not to be associated with a high incidence of ischaemic heart disease (Brunzell et al 1976) and it is uncommon among patients with acute myocardial infarction and their relatives (Gordon and Kannel 1976).

A prospective study of 1648 Finnish men aged 50 - 53 years, however, demonstrated a significant independent correlation between triglyceride levels in excess of 1.7 mmol/L and coronary heart disease mortality (Pelkonen et al 1977).

Reversibility of Risk:

What evidence is there that lowering plasma lipids affects the risk of C.H.D.? Armstrong and Megan (1975) reported regression of experimental atherosclerosis in primates fed a fat modified diet.

Controlled trials of fat - modified diets in the primary prevention of C.H.D. have suggested that the reduction of plasma lipids by controlling diet may be beneficial in the primary prevention of C.H.D. (Dayton et al 1969; Miettinen et al 1972). Results of secondary prevention studies are less encouraging. Hypolipidaemic drugs did not reduce the C.H.D. mortality in the Coronary Drug Project (Coronary Drug Project Research Group 1975) but they were given without regard to the presence or type of plasma lipoprotein disorder. It may be that drug therapy could prove more useful if used only when specifically indicated.

Association with Other "Risk Factors":

A strong inverse relationship exists between plasma HDL level and weight and somewhat weaker associations exist between HDL levels and glucose intolerance and systolic blood pressure (Gordon et al 1977). No significant relationship has been demonstrated with either cigarette smoking or left ventricular hypertrophy.

Mechanism of Risk:

The lipids found in fibrolipid atheromatous plaques consist essentially of cholesterol and certain other steroids, phospholipids and a small amount of triglyceride. These lipids are of course also present in lesion-free areas of arterial intima and there has been considerable debate as to how and why cholesterol, in particular, accumulates in the vessel walls.

Preformed cholesterol is transported in the plasma attached to very low density (VLDL), low density (LDL) or high density (HDL) lipoprotein. VLDL is catabolized within the circulation and its metabolites returned to the liver, but both LDL and HDL are transported across the vascular endothelium by a process of micropinocytosis (Stein and Stein 1973). LDL carries some twenty times more cholesterol than HDL and is taken more easily into the intimal cells where it is degraded by lysozymes and deposits its cholesterol. HDL accounts for only a small proportion of intimal cholesterol (Smith 1974).

Recent studies suggest that HDL may constitute a factor which in fact inhibits the net accumulation of lipid in the intima (Eriksson and Carlson 1973; Miller and Miller 1975). Bondjers and Bjorkerud (1973) demonstrated that when atherosclerotic rabbit aorta was incubated in HDL the cholesterol content of the lesions fell. It seems likely that HDL exerts an opposite effect to LDL in that it promotes cholesterol retrieval from the same cells.

Thus there are experimental findings to explain some of the clinical correlations which have been described: premenopausal females have higher HDL levels than men, regular exercise is associated with increases in HDL, and the Framingham, Tromso and Israeli groups observations that HDL is inversely related to C.H.D. morbidity. (Gordon et al 1977; Miller et al 1977; Goldbourt and Medalie 1979).

Other Dietary Factors:

In 1977, Morris et al reported that CHD incidence was highest in those taking the lowest intake of dietary fibre and that CHD incidence was inversely associated with total energy intake. A high extraction rate of flour results in reduced levels of essential fatty acids (Sinclair 1977).

Oliver (1979) has suggested how essential fatty acids may play an important role in the synthesis of different prostaglandin derivatives and thus on the balance between platelet aggregating and anti-aggregating influences and possible thrombogenic mechanisms. There is good evidence that vegeterians have a lower incidence of CHD than non-vegetarians (Philips et al 1978) but this may not be related to diet alone since vegetarians almost certainly differ from non-vegetarians in other characteristics.

OTHER "RISK FACTORS".

Many other factors have been reported as being important in the pathogenesis of C.H.D. In endocrine disorders, such as diabetes mellitus, various metabolic factors, including hyper-lipidaemia, obesity and perhaps hypertension, may combine to put an individual at risk. To further complicate matters, antidiabetic therapy, diet, oral agents and insulin have all at some time been accused of increasing the risk (Joint Working Party Report 1976).

The association between atherosclerosis and obesity is still controversial. Obesity is usually associated with the presence of other "risk factors" such as hypertension, diabetes mellitus, or elevated plasma lipid levels and it is suggested that obesity is important as a risk mainly because of these associations. The consensus of opinion seems to be that excess body weight should be considered as a relatively low ranked "risk factor". (Pelkonen et al 1977).

The role of physical activity and personality as independent "risk factors" of C.H.D. have been widely examined, but these have proven extremely difficult to evaluate.

The oral contraceptive pill has been accused of causing a risk of C.H.D. In under 45 year olds the majority of pill users who develop clinical C.H.D. have at least one other "risk factor" - hyperlipidaemia, hypertension or cigarette smoking. It is probable that

oral contraceptives add significantly to the risk of developing C.H.D. only in those already at increased risk.

CONCLUSION.

The risk of developing C.H.D. is increased in certain identifiable groups of individuals. Some of those factors associated with an increased risk of C.H.D. cannot be modified. Of those "risk factors" which are modifiable, hypertension, blood lipid levels and cigarette smoking are generally held to be most important.

Much research has been carried out in an attempt to implicate and to explain the possible implication of these various "risk factors" in the initiation of atherosclerotic lesions be it by endothelial damage or by enhancing the likelihood of abnormal cellular proliferation. Each of these "risk factors" however may also play an important role in the evolution of plaques by increasing the chances of thrombus formation and/or persistence.

C.H.D. mortality has fallen in the USA in the past 15 years and more so than mortality from other causes (Gordon and Thom 1975). Why this should have occurred is difficult to define since the 'American way of life' has apparently changed quite radically over this period with altered dietary, smoking and physical activity habit and improved treatment of hypertension.

REFERENCES.

ARMSTRONG, M.L. & MEGAN, M.B. (1975). Arterial fibrous proteins in Cynomolgus monkeys after atherogenic and regression diets. Circulation Research 36, 256.

ASMUSSEN, I. & KJELDSEN, K. (1975). Intimal ultrastructure of human umbilical arteries. Observations on arteries from newborn children of smoking and non-smoking mothers. Circulation Research 36, 579.

BALL, K. & TURNER, R. (1974). Smoking and the heart. The basis for action. Lancet, 2, 822.

BARR, D.P., ROSS, E.M. & EDER, H.A. (1951). Protein-lipid relationships in human plasma. American Journal of Medicine, 11, 480.

BENDITT, E.P. (1977). The origin of atherosclerosis. Scientific American, 336, 74.

BONDJERS, G. & BJORKERUD, S. (1973) In Atherosclerosis, Ed. by Schettler, A. and Weizel, A. Vol.3, p110, Berlin, Heidleberg, New York, Springer Verlag.

BRUNZELL, J.D., SCHROTT, H.G., MOTULSKY, A.G. & BIERMAN, E.L. (1976). Myocardial infarction in the familial forms of hypertriglyceridaemia. Metabolism Clinical and Experimental 25, 313.

CORONARY DRUG PROJECT RESEARCH GROUP (1975). Clofibrate and Niacin in coronary heart disease. Journal of the American Medical Association 231: 360.

DAYTON, S., PEARCE, M.L., HASHIMOTO, S., DIXON, W.J. & TOMIYASU, U. (1969). A controlled clinical trial of a diet high in unsaturated fat in preventing complications of atherosclerosis. Circulation 39/40, Supplement, 2.

DOLL, R. & HILL, A.B. (1964). Mortality in relation to smoking: ten years observations on British doctors. British Medical Journal 1, 1399, 1460.

DOLL, R. & PETO, R. (1976). Mortality in relation to smoking: 20 years observations on male British doctors. British Medical Journal, 2, 1525.

ERIKSSON, M. & CARLSON, L.A.(1973) In Atherosclerosis. Ed. by Schettler, A. and Weizel, A. Vol.3, p838. Berlin. Heidleberg, New York. Springer Verlag.

FROST, H. (1973) In Atherogenesis, (Ed: by Shimamoto, T., Numano, F., Addison, G.M. Vol.2, p32. Amsterdam, Excerpta Medica.

FRY, D.L. (1973) In Atherogenesis initiating factors. Aba Foundation Symposium No.12. New Series. (Ed. by Porter, R. Knight J. p.93 Amsterdam, Excerpta Medica.

GOFMAN, J.W., YOUNG, W., & TANDY, R. (1966). Ischaemic heart disease, atherosclerosis and longevity. Circulation 34, 679.

GOLDBOURT, U., & MEDALIE, J.H. (1979). High density lipoprotein cholestrol and incidence of coronary heart disease - the Israeli ischemic heart disease study. American Jounal of Epideminology 109, 296.

GORDON, T., KANNEL, W.B., McGEE, D., & DAWBER, T.R.(1974) Death and coronary attacks in men after giving up cigarette smoking. A report from the Framingham study. Lancet 2, 1345.

GORDON, T., & THOM, T. (1975) The recent decrease in CHD mortality. Preventive Medicine 4: 115.

GORDON, T., & KANNEL, W.B., (1976). Obesity and cardiovascular diseases: the Framingham study.Clinics in endocrinology and metabolism 5. 367.

GORDON, T., CASTELLI, W.P., HJORTLAND, W.C., KANNEL, W.B., & DAWBER, T.R. (1977). High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. American Journal of Medicine, 62, 707.

INTERSOCIETY COMMISSION FOR HEART DISEASE RESOURCES, (1970). Primary prevention of the atherosclerotic diseases. Circulation, 42, A55.

JOINT WORKING PARTY REPORT, ROYAL COLLEGE OF PHYSICIANS OF LONDON and the BRITISH CARDIAC SOCIETY, (1976). Prevention of coronary heart disease. Journal of the Royal College of Physicians, Vol. 10, No.3. p.7.

KANNEL, W.B., CASTELLI, W.P., GORDON, T. & McNAMARA, P.M. (1971). Serum cholesterol, lipoproteins and risk of coronary heart diseases. The Framingham study. Annals of Internal Medicine, 74, 1.

KANNEL, W.B., & DAWBER, T.R. (1974). Hypertension as an ingredient of a cardiovascular risk profile. British Journal of Hospital Medicine, 11, p.508.

KEYS, A., ARAVANIS, C., BLACKBURN, H., VAN BUCHEM FSP, BULZINAR, DJORDJEVIC, BS, FIDANZA, F, KARVONEN, MJ, MENOTTI, A., PUDDU. V. & TAYLOR, H.L. (1972). Probability of middle aged men developing coronary heart disease in five years. Circulation 45, 815.

KEYS, A., & FIDANZA, F. (1960). Serum cholesterol and relative body weight of coronary patients in different populations. Circulation 22, 1031.

MEITTINEN, M., TURPEINEN, O., KARVONEN, M.J., ELOSULO, R., & PAAVILAINEN, E. (1972). Effect of a cholesterol lowering diet on mortaility from coronary heart disease and other causes. Lancet, 2, 835.

MILLER, G.J., & MILLER, N.E. (1975). Plasma high density lipoprotein concentration and development of ischaemic heart disease. Lancet, 1, 16.

MILLER, N.E., FORDE, O.H., THELIE. D.S., & MJOS, O.D., (1977). The Tromso Heart Study. High-density lipoproteins and coronary heart disease: a propsective case-control study. Lancet, 1, 965.

MORRIS, J.N., MARR, J.W., & CLAYTON, D.G., (1977). Diet and heart: a postscript. British Medical Journal 2: 1307.

NIKKILA, E. (1953). Studies on the lipid-protein relationship in normal and pathologic sera and the effect of heparin on serum lipoproteins. Scandinavian Journal of Clinical and Laboratory Investigation. 5, (suppl.) 8.

OLIVER, M.F., & BOYD, G.S. (1955). Serum lipoprotein patterns in coronary sclerosis and associated conditions. British Heart Journal. 17, 299.

OLIVER, M.F. (1979). Fats and atheroma. British Medical Journal. 889-890.

PELKONEN, R., NIKKILA, E.A., KOSKINEN, S., PENTTINEN, K., & SARNA, S. (1977). Association of serum lipids and obesity with cardiovascular mortality. British Medical Journal, 2, 1185.

PHILIPS, R.L., LEMON, F.R., BEESON, W.I., & KUZMA, J.W. (1978). Coronary heart disease mortality among Seventh Day Adventists with differing dietary habits; a preliminary report. American Journal of Clinical Nutrition 31, 191.

SINCLAIR, H. (1977). Diet and heart disease. British Medical Journal. 2, 1602.

SMITH, E.B. (1974). In Advances in Lipid Research, Ed. by Paoletti, R., Kritchevsky, D. Vol.12, p1. New York, London, Academic Press.

STEIN, Y., & STEIN, O. (1973). In Atherogenesis: Initiating factors. Ciba Foundation Symposium, Number 12. New series Ed. by Porter, R., Knight, J. p.165 Amsterdam, Exerpta Medica.

TAYLOR, S.H., PEARSON, M.C., & MYINT, S. (1974). Circulatory and metabolic effects of smoking cigarettes with and without tobacco substitute and their modulation by beta receptor blockade. Quarterly Journal of Medicine,

TEXON, M. (1977). The haemodynamic basis of atherosclerosis. In Atherosclerosis. Ed. by: Manning G.W., Haust M.D. p.180. New York, Plenum Press,

VETERANS ADMINISTRATION CO-OPERATIVE STUDY GROUP (1970) Effects of treatment on morbidity in hypertension. Journal of the American Medical Association, 213, 1152.

WILHELMSSON, C., VEDIN, J.A., ELMFELOT, D., TIBBLEN, G., & WILHELMSEN, L. (1975). Smoking and myocardial infarction. Lancet, 1, 415.

CHAPTER III.

THE FIBRINOLYTIC SYSTEM.
BASIC COMPONENTS AND MECHANISMS.

INTRODUCTION.

Many proteolytic enzymes are capable of digesting fibrin but the term "fibrinolysis" is, by convention, usually confined to the specific process of fibrin degradation caused by the splitting of a limited number of peptide bonds by plasmin generated in the plasminogen-plasmin system. In essence this fibrinolytic system consists of a number of elements which either promote or inhibit the conversion of a polypeptide proenzyme, plasminogen, to the active serine protease, plasmin. Plasmin has a broad substrate specificity but is rendered relatively specific for fibrin in vivo by the nature of the molecular biology of the fibrin, plasminogen, plasmin and antiplasmin interactions. This specific and limited process allows clot dissolution to occur without the risk of widespread proteolysis of other proteins throughout the body.

In this chapter current concepts regarding the basic components and mechanisms of fibrinolysis and its induction will be reviewed. Since the literature on the

fibrinolytic system is vast, this review must of necessity be selective.

HISTORICAL BACKGROUND.

Probably because it is seldom obtrusive enough to attract attention, it is only within the past quarter century that fibrinolysis has been appreciated as the manifestation of a co-ordinated physiological system.

Fibrinolysis has however been known as a phenomenon for about 200 years. As long ago as 1794 Hunter recorded that in animals chased to exhaustion, the blood remained fluid in the vessels and did not clot when it was removed from the body. It was however not until the beginning of this century that Morawitz (1906) provided a partial explanation for Hunter's observations when he discovered that the blood from sudden-death victims contained no fibrinogen and further that blood from these victims destroyed fibrinogen and fibrin in normal blood. Morawitz suggested that these phenomena were the result of enzymatic activity (1906).

Spontaneous dissolution of fibrin clots in vitro was observed by a number of authors. Denis (1838) observed that the blood clots obtained in "wet cupping" redissolved within a day and Green (1887) demonstrated that fibrin, prepared from ox blood, dissolved when incubated in saline and could not by "re-clotted" by thrombin. The term fibrinolysis appears to have been coined by Dastre (1895), who observed that fibrin lysed when left in contact with

blood from dogs who had been repeatedly bled. Some localisation of the source of this spontaneous in vitro fibrin lysis was obtained when Hedin (1904) reported fibrinolytic activity in the globulin fraction of ox blood.

Towards the end of the nineteenth century it became obvious that not only was fibrin lysed spontaneously in vitro but that lytic activity could be induced in vitro. Proteolytic and fibrinolytic activity was observed in chloroform treated serum by Denys and Marbaix (1889), and it was shown by Delezenne and Pozerski (1903) that chloroform induced fibrinolytic activity probably by removing fibrinolytic inhibitors. Opie and Barker (1907) found that the proteolytic activity induced in serum in vitro by chloroform was, like the spontaneous fibrinolytic activity, in the globulin fraction.

At about the same time, Nolf (1908) described experiments in which he induced fibrinolytic activity in vivo in dogs by means of intravenous injections of peptones. He considered that the lysis of blood clots was the normal end-result of the coagulation process and, like coagulation, fibrinolysis was due to proteolytic enzymes. He conjectured further that the linings of blood vessels contributed components of the fibrinolytic system and he suggested - seventy years ago - that disturbances of fibrinolysis could result in the deposition of fibrin on the vessel wall.

Thus early in the twentieth century the phenomenon of fibrinolysis was well recognised and its proteolytic

nature fairly well understood. Although the identity of the proteolytic enzyme was unknown, it was realised that the enzyme must normally be inert and require activation. Little further advance was made, however, until a chance observation in the field of bacteriology was made by Tillett and Garner (1933). They noted that culture filtrates of haemolytic streptococci lysed fibrin in human blood clots and they attributed this activity to the production by these organisms of a fibrinolytic enzyme "streptococcal fibrinolysin" (streptokinase). It soon became evident that the streptococcal factor per se was not the fibrinolytic agent. In 1941 Milstone reported that streptokinase could not lyse purified human fibrin but if trace amounts of the euglobulin fraction of human serum were added to the fibrin, rapid lysis ensued.

Christensen (1945) and Kaplan (1944) demonstrated that the serum factor was a proenzyme which was activated to a proteolytic enzyme. The proenzyme was named "plasminogen" and the protease "plasmin".

In the past decade our knowledge about plasminogen, its chemistry and interactions has increased greatly. Studies on the structure of plasminogen and plasmin have led to elucidation of their complete primary structure. With this knowledge, understanding of phenomena such as the activation of plasminogen has become possible. The references mentioned above are quoted by Fearnley (1965) and Marsh (1981) in review articles.

In the past five years a further major advance was made when three separate research groups reported the

discovery and characterisation of a new and physiologically most important inhibitor of plasmin in human blood, the so-called α_2 antiplasmin (Mullertz and Clemmensen 1976; Collen 1976; Moroi and Aoki 1976).

PLASMINOGEN.

Purification and Structure of Plasminogen.

Native plasminogen is a single chain β globulin composed of 791 amino acid residues and about 2% carbohydrate. It is not known what the functional purpose of the carbohydrate is but it contributes significantly to the electrophoretic heterogeneity of the molecule (Sottrup-Jensen et al 1978.) The structure of human plasminogen was originally described by Robbins and his co-workers and its physicochemical properties reviewed by them in 1970 (Robbins and Summaria 1970). However, since then our knowledge of the molecular structure and function of plasminogen has advanced significantly.

Several methods have been used to purify human plasminogen. The plasminogens originally described by Robbins et al; (Robbins et al 1965, 1972; Summaria et al 1972) were obtained from Cohn fraction $III_{2,3}$ and had an NH_2-terminal lysine (or valine) and isoelectric points between pH 6.4 and pH 8.5. Other workers were able to produce similar preparations of plasminogen (Liu and Mertz 1971;)

Wallen and Wiman (1970, 1972) were able to show that

the plasminogen obtained from Cohn fraction III could be separated into two groups of differing molecular form, one group,

a) with NH_2-terminal glutamic acid and isoelectric points between pH 6.0 and pH 6.6 and a second group,

b) with NH_2-terminal lysine or valine and isoelectric points between pH 7.3 and pH 8.8.

The electrophoretic mobility of these two groups of plasminogen on starch gel is different, the NH_2-glutamic acid group having mobility similar to that of plasminogen in unfractionated plasma.

Wallen and Wiman (1970, 1972) suggested that the NH_2-terminal glutamic acid plasminogens (glu-plasminogens) are those normally present in circulating plasma and the NH_2-terminal lysine (or valine) forms (lys-plasminogens) are the result of partial proteolytic degradation of native glu-plasminogen.

Using an affinity chromatographic technique on lysine substituted agarose (Deutsch and Mertz 1970) in the presence of plasmin inhibitor (Aprotinin) Collen et al (1972a, 1975) obtained plasminogen from fresh frozen plasma. This plasminogen was almost exclusively glu-plasminogen and had isoelectric points between pH 6.1 and 7.1 with a molecular weight (on SDS gel electrophoresis) of 90,800 daltons. When the plasmin inhibitor was omitted during the chromatography of fresh frozen plasma, molecular forms with NH_2-terminal lysine, isoelectric points between pH 6.5 and 8.4 and molecular weights of

84,000 daltons were also obtained. Claeys et al (1973) demonstrated that glu-plasminogen could be converted to lys-plasminogen by limited digestion with plasmin and other workers (Rickli and Otavsky 1973; Wiman and Wallen 1973; Walther et al 1974) have shown that lys-plasminogen is detectable transiently during the activation of glu-plasminogen with urokinase.

As will be described later lys-plasminogen is derived from glu-plasminogen by the release of peptides from the NH_2-terminal of the molecule. Glu-plasminogen and lys-plasminogen differ from each other with regard to chemical and functional properties. Apart from molecular weight there are other differences in the properties of glu-plasminogen and lys-plasminogen. Infused glu-plasminogen has a longer half life than lys-plasminogen (Collen et al 1972b). Lys-plasminogen is activated more rapidly than glu-plasminogen (Wallen and Wiman 1975) and the lys form is more readily adsorbed to fibrin (Thorsen 1975). Both types of plasminogen occur in multiple electrophoretically separable forms, perhaps due in part to differences in sialic acid content. (Collen and de Maeyer 1975).

The complete amino acid sequence of plasminogen is known (Sottrup-Jensen et al 1978, Wiman and Wallen 1975a). The molecule is made up of three distinct regions which are relevant to the activation process,

a) the preactivation peptides (residues 1-76)

b) the A (heavy) region which contains five kringle structures (residues 77-560)

and c) the B (light) region (residues 561-790) which contains the active site of plasmin.

The kringles contain the critical lysine binding sites which are competed for, in the physiological situation, by fibrin and α_2 antiplasmin.

For details of the molecular structure of plasminogens and their physicochemical properties the reader is referred to review articles by Wallen (1978, 1980) and Wiman and Wallen (1975b).

Assay of Plasminogen.

Plasminogen can be assayed indirectly by measuring plasmin activity after activation of plasminogen with urokinase or streptokinase or it can be assayed using quantitative immunologic techniques.

Many substrates have been used for measuring plasmin activity. These include casein (Johnson et al 1969), fibrin, (Brakman 1967) and synthetic substrates such as esters of arginine and lysine (Troll et al 1954). In recent years chromogenic substrates including in particular a rather specific plasmin substrate (S-2251, Kabi Diagnostica, Stockholm) have been used. (Soria et al 1978, Friberger et al 1978).

However, it has to be realised that assaying plasminogen as plasmin activity after activation is influenced by the rate of activation of different plasminogens and by the fact that plasmin autodigestion will intertere with the result (Gaffney et al 1977).

Furthermore, measurement of plasminogen as plasmin activity is influenced by the presence of plasmin inhibitors and these inhibitors must be inactivated (Alkjaersig 1959) or, preferably, the plasminogen selectively precipitated (Brakman 1967, Hedner et al 1966).

Plasmin inhibitors do not interfere with plasminogen determinations using immunochemical methods. Various immunologic methods have been described including radial immuno diffusion (Mancini et al 1965) electroimmuno diffusion (Laurell 1966) and radioimmuno assay (Rabiner et al 1969). However, immunologic assays reflect only the amount of antigen present. They do not measure biological activity and they tend to give an over estimate of the activatable plasminogen present.

Plasminogen Levels.

Different methods of assaying plasminogen have yielded different normal ranges for the plasminogen level of human plasma. (Rabiner et al 1969; Sherry 1968, Heimburger 1971).

Plasminogen levels are low at birth, particularly in premature babies in whom very low plasminogen levels are thought to be an important factor in the development of the hyaline membrane disease. Low levels are also found in patients with advanced hepatic cirrhosis and in disseminated intravascular coagulation. A rapid reduction of plasminogen levels occurs during thrombolytic therapy.

It would appear that plasminogen acts as an acute phase reactant, high levels being recorded following trauma, post-operatively, during acute infections and after myocardial infarction. (Lackner and Javid 1973) Plasminogen levels do not appear to be influenced by sex differences and do not exhibit diurnal variation. (Brakman et al 1966; Rosing et al 1970). Levels do, however, rise during the latter months of pregnancy (Bonnar et al 1969) and in women taking oestrogen containing contraceptives (Lackner and Javid 1973).

Origin and Metabolism of Plasminogen.

To date the primary site of plasminogen synthesis has not been firmly established for any species. The liver, bone marrow eosinophils and kidney have all been implicated at some time (Miller and Bale 1954; Barnhart and Riddel 1963; Highsmith and Kline 1971). It appears that considerable species variation must operate but more work is required to resolve the complex problem of the source of plasminogen molecules.

The metabolic fate of human plasminogen bearing a radioactive iodine label has been studied. (Collen et al 1972b). The plasma radioactivity declined logarithmically. The plasma radioactivity half life was found to be 2.21 $\pm$ 0.29 days and it was calculated that the absolute catabolic rate of plasminogen was 4.8 $\pm$ 1.2 mg/kg/day. Collen also demonstrated that while NH_2-terminal glutamic acid plasminogen has a half life of 2.0-

2.5 days, NH_2-terminal lysine or valine plasminogen has a half life of less than 1 day.

Lysine Binding Sites.

The discovery of the antifibrinolytic effect of ε - aminocaproic acid (EACA) was of great importance for the study of the fibrinolytic system. EACA and other subsequently discovered antifibrinolytic agents, trans-aminomethyl cyclohexane-1-carboxylic acid (tranexamic acid) and p-aminobenzoic acid (PAMBA) possess a basic and an acidic group within a certain distance of each other. Lysine also belongs to this group of compounds although its effect on the fibrinolytic system is much weaker.

Lysine and the antifibrinolytic agents above interact significantly with one or more binding sites on the plasminogen molecule - the so-called lysine binding sites - (LBS). The location of some of these LBS in the primary structure of plasminogen is known. (Rickli and Otavsky 1975; Sottrup-Jensen et al 1978).

The importance however of the discovery of these LBS lies in the role that they play in the binding of plasminogen to fibrin. It may be that the differences in binding of glu-plasminogen and lys-plasminogen to fibrin are due to a masking effect by the NH_2-terminal region of the glu-plasminogen of the relevant LBS.

It has been shown that a LBS mediates the interaction of plasminogen with the inhibitor α_2 antiplasmin (Christensen and Clemmensen 1977; Wiman and Collen 1978a).

α 2 antiplasmin competitively affects the binding of plasminogen to fibrin but the full expression of α 2 antiplasmin binding takes place only with plasmin. Relatively little (about 30%) of the α 2 antiplasmin is complexed to plasminogen in vivo but this weak binding is enough to explain the poor binding of both lys and glu-plasminogens to fibrin in plasma. LBS are involved in both reactions.

PLASMIN.

Activity.

Plasmin is a non-specific proteolytic enzyme capable of hydrolysing many different proteins. Plasmin cleaves susceptible lysyl and arginyl bonds and splits synthetic esters and amides of the basic amino acids lysine and arginine (Troll et al 1954).

Studies of the plasmin degradation of fibrinogen have shown that plasmin cleaves about 60% of the lysyl and arginyl bonds. This is in contrast to thrombin which cleaves only four arginyl bonds in the fibrinogen molecule and trypsin which splits almost all of the arginyl and lysyl bonds of fibrinogen. Although its principal targets are fibrin and fibrinogen, plasmin can also hydrolyse a number of other plasma proteins including prothrombin and coagulation factors V and VIII. By activating factor XII, plasmin exerts an influence not only on the coagulation system, but also on the kallikrein-kinin and complement

systems. It can also directly activate factor VII and the components of the complement system. Plasmin induces platelet aggregation and release. (Stormorken 1975).

Assay of Plasmin.

Plasmin is not normally detectable in plasma, probably because it is rapidly neutralised by an excess of inhibitors. As already described in the subsection on the assay of plasminogen, plasmin activity can be measured on a variety of substrates including, caesin, fibrin, esters of lysine and arginine and synthetic chromogenic substrates.

Structure of Plasmin.

Plasmin is produced from plasminogen by limited proteolytic cleavage accompanied by conformational changes in a process similar to the formation of other proteolytic enzymes (e.g. thrombin, trypsin and activated factors IX and X). It is composed of two polypeptide chains of different size connected by disulphide bonds. The heavier chain has been called the A chain and the lighter chain the B chain. The active centre of plasmin has been localised to the B chain. (Summaria et al 1967).

THE ACTIVATION OF PLASMINOGEN.

Activation of proteolytic enzymes from their proenzymes usually involves a limited proteolytic cleavage of the zymogen molecule. During this process, cleavage products, usually peptide fragments, are released. Scission of the polypeptide chain(s) of the proenzyme allows conformational changes to occur thus permitting the formation of the active centre and the generation of enzymatic activity.

Most studies on the activation of human plasminogen have been carried out in purified systems using urokinase as the activator and it is results from these studies which will be described first.

Two sites in the native glu-plasminogen molecule appear to be of particular importance with regard to its activation. One is in the NH_2 terminal where three bonds (Arg^{68} - Met^{69} ; Lys^{77} - Lys^{78} and Lys^{78} - Val_{79}) are sensitive to proteolytic cleavage. Cleavage of these bonds gives rise to the release of peptide materials (the so-called activation or preactivation peptides) with the formation of lys-plasminogen. (Rickli and Otavsky 1973; Walther et al 1974, Wiman and Wallen 1973). The other site of particular interest is an Arg-Val bond approximately 600 residues from the NH_2 terminus of glu-plasminogen. The cleavage of this bond results in the formation of plasmin.

Release of peptide material from the NH_2 terminus of glu-plasminogen is succeeded by conformational changes in the remaining molecule (Sjoholm et al 1973), which facilitate the further cleavage of plasminogen to the two chain plasmin.

There has been some disagreement regarding the molecular events which occur during the activation of glu-plasminogen. It used to be held that the activation occurred in distinct steps, the first being the formation of the intermediate lys-plasminogen and the second the generation of plasmin. (Rickli and Otavsky 1973; Walther et al 1974; Wiman and Wallen 1973; Wallen and Wiman 1975).

However, it has since been shown that activation of glu-plasminogen by urokinase in the presence of aprotinin, which inhibits plasmin but not urokinase, results in the formation of plasmin with the preactivation peptide still attached to the A chain - glu-plasmin; (Summaria et al 1975; Violand and Castellino 1976). Thus it seems that the release of the so-called preactivation peptide does not occur during activation of glu-plasminogen by urokinase alone, but that the bonds are cleaved by plasmin. Furthermore, it seems that release of the preactivation peptide is not necessary for the generation of plasmin. Castellino and his co-workers (Violand and Castellino 1976) suggest that the activation of plasminogen can therefore follow two paths:

a) The major path - catalytic amounts of plasmin cleave the bonds which release the preactivation

peptide converting glu-plasminogen to lys-plasminogen which is rapidly activated by urokinase to lys-plasmin, and

b) Urokinase converts glu-plasminogen to glu-plasmin which may in turn be converted to lys-plasmin.

Using urokinase as the activator it can be shown that lys-plasminogen is activated about 10 - 20 times faster than glu-plasminogen. (Wallen and Wiman 1975; Claeys and Vermylen 1974).

Studies on plasminogen activation with tissue activators are rather scarce, largely because of the difficulty of obtaining pure tissue activator. In contrast to urokinase, tissue activator is strongly adsorbed to fibrin (Thorsen et al 1972) and it would appear that this interaction with fibrin has a strong stimulating effect on activation of plasminogen.

PLASMINOGEN ACTIVATORS.

Activators of plasminogen are distributed widely throughout the body but clear understanding of the molecular biology, the differentiation and the possible inter-relationships of these plasminogen activators is limited. At present there is no single universally accepted classification.

Plasminogen activators are found in most body tissues and in most body fluids including blood, urine, saliva, tears and cerebrospinal fluid. Certain bacterial strains, in particular streptococci, produce substances which activate plasminogen and activators can be produced in tissue cultures.

Tissue Activators.

The fibrinolytic activity of tissues is caused by a cellular activator of plasminogen. The origin of this discovery (Astrup and Permin 1947) depends on observations of the liquefaction of plasma clots used as substrates in the cultivation of explanted tissue cells. Early workers found that in vitro cultures of certain tissues or cells, in particular epithelial tissues or tumour cells, liquefied the supporting plasma clot.

The distribution of plasminogen activator in tissues varies from organ to organ and from species to species. All human organs, with the exception of normal liver, contain plasminogen activator (Tagnon and Palade 1950; Lewis and Ferguson 1950; Albrechtsen 1957). Some organs and tissues are always found to have high activator content while others tend always to have a low content. The uterus is highly active, with cyclic fluctuations of endometrial activity. Human prostate, and adrenals, also tend to contain high concentrations of tissue activator while the testes and spleen have relatively little

activator content (for review See Astrup 1978).

Two forms of tissue activator have been described - a tissue activator which can be extracted in small quantities using saline and a tissue activator which can be extracted in much greater quantities using strong solutions of potassium thiocyanate. It may be that transformation between these two forms is possible (Astrup 1978).

Using a histochemical slide technique (Todd 1959) it can be shown that most tissue activator is present in the endothelium of small veins and that the fibrinolytic activity of tissues is related to their vascularity.

In studies on the location of plasminogen activator in subcellular fractions, both the lysosomal and microsomal fractions have been shown to contain activator (Ali and Lack 1965; Nakahara and Celander 1968).

Several workers have purified plasminogen activator from pig heart. (Nakahara and Celander 1968; Bachmann et al 1964; Rickli and Zaugg 1970; Hijikata et al 1974; McCall and Kline 1965; Cole and Bachmann 1977) and plasminogen activator has been partially purified from pregnant hog ovaries (Kok and Astrup 1969).

The activity of tissue plasminogen activators suggests that they must have an important physiological role. The true nature of this role is not clear but presumably they are important in the lysis of extravascular fibrin and in what Astrup (1977) has termed the processes of "physiological tissue repair".

Urokinase.

Human urine is able to liquefy fibrin clots. Although urine contains a number of proteases, the fibrinolytic activity originally described by Macfarlane and Pilling in 1947 is distinct from non-specific proteolysis and is due to the presence of an activator of plasminogen. This activator in urine was termed urokinase by Sobel (Sobel et al 1952).

Since its discovery, urokinase has been widely studied and its excretion, purification and properties widely examined. The origin of urokinase has been a matter of some dispute. Originally it was assumed that the urinary activator originated in the blood and that it represented a plasma activator excreted by the kidney.

There is now abundant evidence that urokinase is not identical to the circulating or vascular activator. More than ten years ago Kuciniski et al (1968) showed that urokinase and plasma activator were immunologically different and Aoki and von Kaulla (1971) have demonstrated that not only are urokinase and plasma activator immunologically different but they also differ in the rates at which they hydrolyse N acetyl L lysine methyl ester (ALMe).

Furthermore, there is evidence that urokinase is actually produced in the kidney.

Later, Painter and Charles (1962) used primary cultures of kidney cells to produce plasminogen activator

and demonstrated that the activator produced migrated on starch gels as urokinase.

Biochemical and immunological studies have shown identity between kidney cell culture activator and urokinase. (Barlow and Lazer 1972, Kucinski et al 1968; Bernik et al 1974).

A variety of methods of isolating and purifying urokinase have been described. Lesuk et al (1965) obtained urokinase in crystalline form. Ogawa et al (1975) used polyacrylonitrile fibre to absorb urokinase from urine. More recently highly purified preparations of urokinase have been obtained using affinity chromatographic techniques. (Holmberg et al 1976; Soberano et al 1976).

At least two forms of urokinase have been described, one having a molecular weight in the range 47,000 to 54,000 daltons and the other a molecular weight of 31,000-34,000 daltons. It has been suggested that the lower molecular weight form may have been derived from the higher form as a result of proteolytic degradation (Lesuk et al 1967).

Much of the work published on the activation of plasminogen has used urokinase as the activator and, as already described, urokinase activates plasminogen by cleaving lysine or arginine bonds.

Leukocyte Plasminogen Activator.

Polymorph leukocytes contain an activator of plasminogen (Koptitar et al 1971; Granelli - Piperno et al

1978) and also produce non-plasmin proteases which are themselves fibrinolytic (Plow and Edgington 1975).

Erythrocyte Plasminogen Activator.

Erythrokinase, an activator of plasminogen, has been partially purified from human erythrocytes (Semar et al 1969).

Plasminogen Activator in Biological Fluids.

Milk, tears and seminal fluid all have plasminogen activator activity but the biochemical nature of the activators involved is not known. (Ogston 1977).

Human and animal bile contain an activator of plasminogen. The activator from bovine bile has been purified. (Oshiba and Ariga 1975). It has a molecular weight of 58,000 daltons and is biochemically and immunologically different from urokinase.

Plasminogen Activators in Neoplastic Cells.

Recently Reich and his co-workers have demonstrated that malignant cells in culture contain elevated levels of plasminogen activators (Reich 1975). Fibrinolytic activity has been found in primary cultures of chemically induced mouse and rat tumours, a variety of human and animal tumour cell lines and in mammalian and avian fibroblasts transformed to malignancy either by chemical

carcinogens or by oncogenic viruses.

Blood Activators.

The origin and mechanism of the development of fibrinolytic activity in the blood has been the subject of much confusion, but recently considerable advances have been made in understanding the nature of plasma fibrinolytic activity. It is now clear that blood can become fibrinolytically active by two different mechanisms :an extrinsic activation system and an intrinsic activation system.

This classification into intrinsic and extrinsic pathways closely parallels the functional division of susceptibility of plasma plasminogen activators to inhibition by C_1 inactivator (Kluft 1978). The intrinsic plasminogen activator pathways are inhibited by C_1 inactivator; the extrinsic pathways are not.

The extrinsic system is represented by the previously named "vascular activator", which is released into the blood from the endothelial cells lining the small vessels, in particular, the small veins (Todd 1959). Using immunofluorescent techniques, Todd and Hargreaves (1975) have shown that vascular activator is located in the vascular endothelium in a similar distribution to that of factor VIII antigen. In the matter of histologic localisation, Warren and Khan (1974) using fibrin autography in conjunction with electron microscopy, showed that the lysis intiated by the endothelial cells seems to

begin at superficial vesicles within the endothelial cytoplasm. It appears that this type of activator is normally not only stored in the endothelial cells but is also actually produced there.

The mechanism by which this vascular activator is released into the circulation remains obsure but will be discussed briefly in Chapter IV.

The intrinsic fibrinolytic system also remains incompletely characterized but seems to include two or more distinct pathways. One of these pathways, the factor-XII-dependent pathway has been extensively studied, but it is only very recently that the various interactions involved in it have been elucidated.

Ratnoff and his co-workers (Ratnoff and Colopy 1955; Ratnoff and Rosenblum 1958) were the first to describe the presence in plasma of an enzyme precursor (Hageman factor) which, when adsorbed onto glass or kaolin, initiated a chain of reactions culminating in blood clotting. Soon thereafter it was discovered that activation of Hageman factor also results in the generation of fibrinolytic activity in normal plasma. (Niewiarowski and Prou-Wartelle 1959; Iatridis and Ferguson 1961).

According to Kaplan et al (1977) activation of the Hageman factor dependent pathways is initiated by the binding of Hageman factor (molecular weight, 80,000 daltons) and a complex of prekallikrein and high molecular weight (HMW) kininogen (280,000 daltons) to negatively charged surfaces. A reciprocal reaction proceeds in which activated Hageman factor converts prekallikrein to

kallikrein, and kallikrein activates Hageman factor. HMW kininogen is a co-factor for both of these reactions. The active site of Hageman factor is generated on activation by kallikrein, and the activated Hageman factor is subsequently cleaved to liberate active Hageman factor fragments (molecular weight 28,000 daltons). Activated Hageman factor cleaves prekallikrein to yield kallikrein which consists of a heavy chain (molecular weight 52,000 daltons) disulfide linked to a light chain (molecular weight, 36,000, or 33,000 daltons) with an active site on the light chain.

Kallikrein activates plasminogen to form plasmin. Digestion of prekallikrein by kallikrein yields proenzymes that appear to represent the previously described proactivator. (Kaplan and Austen 1972).

Factor XI circulates bound to HMW kininogen and is activated by activated Hageman factor in the presence of HMW kininogen. The complex of activated factor XI and HMW kininogen activates Hageman factor. Thus, factor XI seems to contribute to the gradual activation of fibrinolytic activity in prekallikrein-deficient plasma. Kaplan et al (1977) also suggest that activated factor XI has a role as a plasminogen activator.

A Hageman-factor-dependent co-factor that functions in fibrinolysis was reported by Ogston et al (1969). Yecies et al (1978) suggest that this co-factor is not a distinct entity but represents the requirement for prekallikrein in the activation of Hageman factor.

Although it is agreed that kallikrein can activate plasminogen, Kluft (1977) has reported that it is responsible for only about 10%-15% of resting fibrinolytic activity and that there is another separate factor-XII-dependent activator.

In addition to the factor-XII-dependent pathway, a blood activator system known as the "peptone pathway" exists. This pathway is effective even in the absence of factor XII (Astrup and Rosa 1974; Schreiber and Austin 1974; Coccheri et al 1978). It can be activated by various organic acids such as fatty acids, higher alkyl sulfates, organic phosphates, inorganic polyphosphates, and acid polysaccharides such as dextran sulphate (Astrup and Rosa 1974).

Clearly many questions remain to be answered before we fully understand how plasminogen is activated in plasma. It is probably not surprising that several pathways have been found in vitro. More than one protease may be capable of cleaving peptide bonds in plasminogen to produce the active enzyme plasmin. Which activators are important in vivo will depend on a number of factors including their kinetic properties, interaction with inhibitors present in plasma and affinities for plasminogen and fibrin. Further work is necessary to find out whether there is a single preferred pathway in vivo or whether multiple pathways exist so that the absence of one factor may be of limited consequence for normal fibrinolytic function.

Methods for assaying plasminogen activators are discussed in Chapter IV.

INHIBITORS OF FIBRINOLYSIS.

In order that widespread proteolysis does not result from the activation of proteolytic enzymes, a complex system of antiproteases has evolved. In blood these antiproteases are directed against the various proteolytic products that may be generated not only by the fibrinolytic system but also by the coagulation system, the complement system and the kallikrein system.

Inhibition of fibrinolysis can in theory occur at two distinct levels: at the level of plasminogen activation (antiactivators) and at the level of active plasmin (antiplasmins).

Antiplasmins.

In the past at least five well defined plasma proteins have been described as having antiplasmin activity in purified systems. These were α_2 macroglobulin, α_1 antitrypsin, inter-α-trypsin inhibitor, antithrombin III-heparin complex and C_1 inactivator. For many years it was widely held that there were essentially two functionally important plasmin inhibitors in plasma; α_2 macroglobulin which was responsible for fast-reacting plasmin inhibition and α_1

antitrypsin which was a slow-reacting antiplasmin.

It is however now known that the major antiplasmin activity in plasma is due to neither of these antiproteases but is due to the more recently described very fast reacting α_2 antiplasmin (Collen 1976; Moroi and Aoki 1976; Mullertz and Clemmensen 1976). α_2 macroglobulin is responsible for inhibiting plasmin formed in excess of that which fully saturates the available α_2 antiplasmin and in the presence of these two antiproteases, other plasma protease inhibitors are not important in the inhibition of plasmin (Collen 1976; Mullertz and Clemmensen 1976)

α_2 Antiplasmin.

Several methods have been used to purify α_2 antiplasmin (Moroi and Aoki 1976; Wiman and Collen 1977; Mullertz and Clemmensen 1976) and it has been shown to be a single-chain polypeptide with a molecular weight of 65,000-70,000 daltons (Wiman and Collen 1977). On electrophoresis it migrates as an α_2 globulin. The amino acid composition of α_2 antiplasmin has been reported (Wiman and Collen 1977; Moroi and Aoki 1976) and it has been shown to be immunochemically different from α_1 antitrypsin, α_2 macroglobulin, C_1 esterase inhibitor, antithrombin III, α_1 antichymotrypsin and inter-α - trypsin inhibitor.

α_2 antiplasmin can be quantitated accurately in plasma using either enzymatic assays based on its very

fast inhibition of plasmin (Edy et al 1978, Teger-Nilsson et al 1977) or immunochemical assays using specific antisera. The normal level of α_2 antiplasmin in plasma is around 70 mg/l plasma or 1 μmol/l (Moroi and Aoki 1976, Mullertz and Clemmensen 1976, Wiman and Collen 1977).

In purified systems and in plasma, α_2 antiplasmin forms a 1:1 stoichiometric complex with plasmin (Wiman and Collen 1977; Collen 1976; Mullertz 1974). The complex formed is devoid of protease or esterase activity and cannot be dissociated with denaturing agents.

α_2 antiplasmin reacts strongly with the light B chain of plasmin and weakly with the heavy A chain.

Of the major plasma plasmin inhibitors, α_2 antiplasmin has been shown to be the only one that is significantly reduced in patients with disseminated intravascular coagulation. (Aoki et al 1977). Its concentration may also be reduced in severe liver disease but is normal in patients with cardiovascular, renal or malignant disease. The inhibitor when measured with the enzymatic assay is temporarily exhausted during streptokinase therapy (Verstraete et al 1978; Teger-Nilsson et al 1977) but residual antigen representing complexed or degraded α_2 antiplasmin may be observed with the immunologic assay. α_2 antiplasmin is a weak acute phase reactant (Teger-Nilsson et al 1977).

Since α_2 antiplasmin has an affinity for lysine binding sites (Moroi and Aoki 1977), it interferes with the binding of plasminogen to fibrin. In plasma this

interference is small and α_2 antiplasmin cannot efficiently displace fibrin-bound plasminogen. (Rakoczi et al 1978). α_2 antiplasmin reacts rapidly and irreversibly with plasmin formed in the circulation but plasmin formed on the fibrin surface, which is bound through its lysine binding site, reacts very slowly with antiplasmin.

Plasmin-α_2 antiplasmin complex can be detected as being immunologically distinct from the precursor molecules (Collen and de Cock 1974, 1975). Assay of this plasmin-antiplasmin complex may be an important index of in vivo fibrinolytic antivity (Collen et al 1977).

α 2 Macroglobulin.

For many years it was accepted that α_2 macroglobulin was the fast-acting and most important inhibitor of plasmin in human plasma, but now it is clear that α_2 macroglobulin represents the slower acting plasmin inhibitor of plasma and its role is to inactivate plasmin formed in excess of the inhibitory capacity of α_2 antiplasmin. (Collen 1976; Mullertz 1974).

Several methods have been described for the purification of α_2 macroglobulin and it has been shown to be a glycoprotein of molecular weight 725,000 daltons (Jones et al 1972). It is composed of two half molecules held together by non-covalent bonds. Each half-molecule is composed of two chains of molecular weight approximately 185,000 daltons held together by disulphide bonds.

α_2 macroglobulin is capable of binding not only plasmin but also trypsin, chymotrypsin, thrombin, kallikrein and elastase. Probably the binding of α_2 macroglobulin with proteases is an irreversible process. Physiologically the importance of α_2 macroglobulin apears to be that it "traps" proteases, and inhibition results from steric hindrance of access of substrate to the protease. After bindng, the proteases retain their ability to react with substrates of low molecule weight but not with those of high molecular weight. α_2 macroglobulin-protease complexes are rapidly cleared from the circulation by mononuclear phagocytes.

The level of α_2 macroglobulin in plasma is age dependent being maximal, 4.5g/l, at 1-3 years and falling to adult levels of 2g/l by 25 years (Ganrot and Schersten 1967). Levels are slightly higher in females than males and increased values are found in nephrosis and hepatic cirrhosis. Streptokinase therapy is associated with decreased levels of α_2 macroglobulin and it may take several days after discontinuing thrombolytic therapy for levels to regain normal (Nilehn and Ganrot 1967; Arnesen and Fagerhol 1972).

Other Antiplasmins.

α_1 antitrypsin, antithrombin III and C_1 inactivator all inhibit plasmin in purified systems but in the presence of normal concentrations of α_2 macroglobulin these inhibitors play no role in the neutralization of plasmin formed in vivo (Collen 1976; Mullertz 1974).

Antithrombin III - heparin complex may play a limited role in the inactivation of plasmin in vivo. (Semeraro et al 1978; Collen et al 1978).

Platelets have antiplasmin activity and although in absolute terms the amount is small - only 1-3% of antiplasmin activity (Den Ottolander et al 1967; Ekert et al 1970) - platelet antiplasmin may be important in allowing fibrin formation in the early stages of thrombus stabilisation (Ekert et al 1970;)

Granulocytes possess antiplasmin activity (Wolosowicz and Prokopowicz 1970) as do many other tissues (lung, spleen, liver, pancreas) and body fluids (urine, seminal fluid, amniotic fluid). Mostly these inhibitors are poorly characterised antiproteases of unknown physiological function.

Assay of Antiplasmins.

α_2 antiplasmin:

α_2 antiplasmin may be assayed either biologically or immunologically. The biological assay is a chromogenic substrate assay of residual plasmin when excess plasmin is added to the test plasma. Chromogenic tripeptide substrates (S 2251 - Kabi Molndal) with short reaction times are used for this assay of the very fast reacting α_2 antiplasmin. Assay kits which contain substrate, plasmin and human normal plasma are commercially available (Coatest (R) Kabi, Molndal). The amidolysis of the substrate can either be traced on a recorder (initial rate

method) or read after a set period of time (end point method). The concentration of α_2 antiplasmin is calculated from a standard curve prepared from human normal plasma dilution.

α_2 antiplasmin can be measured immunologically by a number of quantitative immunochemical techniques including radial immunodiffusion, electroimmunoassay or radioimmunoassay. The necessary monospecific antiserum is commercially available. (Nordic Immunological Laboratories, Tilburg, The Netherlands).

The most widely used assay is probably the electroimmunoassay (Laurell 1966).

α_2 Macroglobulin.

α_2 macroglobulin which is responsible for inhibiting plasmin activity which exceeds the inhibiting capacity of α_2 antiplasmin can be assayed either immunologically or biologically.

The immunological assay is probably most conveniently carried out as a radial immunodiffusion assay using commercially produced plates. (Partigen Immunodiffusion plates) but monospecific antisera can be purchased and an electroimmunoassay is also widely used.

The biological activity of α_2 macroglobulin can be estimated from the esterase activity of its complex with trypsin using a synthetic substrate (Ganrot 1966).

Plasmin - α_2 antiplasmin complex.

Antibodies with a greater affinity for the plasmin-α_2 antiplasmin complex than for its precursors can be prepared and using these antisera latex agglutination assays and haemagglutination assays have been described. (Collen and DeCock 1975; Collen et al 1977; Bini and Collen 1978). These antisera are not yet commercially available and it is as yet too soon to evaluate the practical value of what seems likely to prove a useful test.

Antiactivators.

The existence in human blood of physiological inhibitors of the activation of plasminogen has been much disputed. Two major obstacles have impeded elucidation of this problem. Firstly, since there are considerable experimental problems of substrate specificity, it is difficult to demonstrate activation inhibitors in the presence of antiplasmins. Assay systems unaffected or minimally affected by antiplasmins are required to detect antiactivators and unfortunately the widely used clot lysis assay systems are very sensitive to plasmin inhibitors. Chromogenic substrates sensitive to plasminogen activators seem likely to prove an important development in future antiactivator studies.

Secondly, the various antiproteases present in plasma may each have some affinity for plasminogen activators and act in concert in vivo without being specific inhibitors.

In spite of these difficulties there is good evidence that human plasma does contain substances capable of inhibiting the activation of plasminogen.

C_1 inactivator, an α_2 globulin of molecular weight 104,000 daltons inhibits $C1_s$, plasma kallikrein, plasmin, factor XII_a and factor XI_a. Insofar as C_1 inactivator is the main inhibitor of Hageman factor and kallikrein in human plasma, it would seem reasonable to expect it to play a role in the inhibition of the intrinsic plasminogen activator pathways.

α_2 macroglobulin inhibits kallikrein and may therefore be important in the regulation of intrinsic fibrinolysis (Ratnoff et al 1969; McConell 1972). Hedner described an inhibitor of urokinase induced fibrinolysis which she has subsequently shown not to inactivate urokinase in a purified system but to inhibit Factor XIIa. (Hedner and Martinsson 1978).

Antithrombin III in the presence of heparin inhibits activated Factor XII (Stead et al 1976)

Evidence for the existence of specific inhibitors of the extrinsic plasminogen activators is conflicting and at present must be regarded as preliminary and of no practical value in clinical investigation.

In contrast the detection and quantitation of inhibitors directed against exogenous plasminogen

activators - urokinase and streptokinase given for therapeutic purposes - may be of great practical importance in designing treatment schedules and dosages.

Human plasma contains antistreptococcal antibodies which react with and neutralise streptokinase. These antibodies are probably the result of previous infection with beta haemolytic streptococci. The initial dose of streptokinase is generally calculated to include sufficient to neutralise these antibodies in addition to the amount infused to produce fibrinolysis.

Many antiproteases including α_2 antiplasmin, α_1 antitrypsin and α_2 macroglobulin inhibit urokinase slowly. Recently Gallimore (1979) has partially purified a urokinase inhibitor which acts rapidly and is detectable using chromogenic substrate assays but not clot lysis methods where it seems that the presence of fibrin may cause a reversible complex to dissociate.

MECHANISM OF THROMBOLYSIS.

In pure systems, plasmin is an aggressive and non specific protease but normally in vivo plasmin activity is restricted and plasmin acts largely as a fibrinolytic enzyme. The regulation of this restriction is not fully understood but some possible mechanisms have been suggested.

The Sherry Hypothesis.

Professor Sol Sherry and his group proposed a dual-phase scheme for the activation of plasminogen. This theory (Alkjaersig et al 1959) offers a working hypothesis for the mechanism by which plasmin activity is restricted in vivo. It is suggested that, in a system which includes both plasma and thrombus, plasminogen is present in two phases - soluble in the plasma and gel within the thrombus. Activation of plasma plasminogen does not normally result in the production of detectable amounts of plasmin since plasmin formed in the plasma is rapidly inhibited by antiplasmins. In contrast, plasmin formed by plasminogen activator diffusing into a thrombus and activating plasminogen adsorbed to fibrin within it is intimately spatially related to its substrate and the effect of inhibitors is greatly reduced. Plasminogen activation within a thrombus therefore results in lysis of fibrin.

In recent years, however, Sherry's intrinsic clot lysis theory has been criticised. Ogston et al (1966) showed that thrombi have a low plasminogen content and de Witt (1964); Fantl (1962) and Hedner and Nilsson (1965) demonstrated that the plasminogen content of plasma and serum are the same, indicating that plasminogen is not consumed during blood coagulation. Only traces of plasminogen have been demonstrated in clots and thrombi. Recently Rakoczi et al (1978) investigated the adsorption

of plasminogen to fibrin in a whole plasma system using radio-labelled plasminogen. They showed that only about 4% of native plasminogen and 8% of partially degraded plasminogen were specifically adsorbed to fibrin. Thus, it seems evident that large amounts of plasminogen are not, as hypothesised, incorporated into fibrin during clotting. Indeed Chesterman et al (1972) have reported that the amount of plasminogen in a thrombus is insufficient to allow more than about 20 per cent lysis when streptokinase is used as the activator. Further evidence of the necessity of extrinsic plasminogen has been produced by NcNicol et al (1965) who have shown that artificial thrombi perfused with plasminogen activator in saline are not fully lysed unless plasminogen is added to the perfusing solution.

The Ambrus and Markus Hypothesis.

The Ambrus and Markus hypothesis suggests that plasmin-inhibitor complexes may circulate in plasma and dissociate in the presence of fibrin because plasmin has a stronger affinity for fibrin than for its inhibitors. (Ambrus and Markus 1960; Back et al 1961). In this manner substrate specificity is effectively achieved. At present this remains only a hypothesis but Wiman and Collen (1978a), to determine whether initial complexes between plasmin and antiplasmin would dissociate in the presence of fibrin, clotted a mixture of fibrinogen, α_2 antiplasmin and plasmin with thrombin and incubated it at 37°C. They

found no evidence of clot lysis after 24 hours incubation and concluded that plasmin-antiplasmin complexes do not dissociate in the presence of fibrin.

The Sharp, Chesterman and Allington Hypothesis:

As a result of the shortcomings of the two hypothesis just described, Chesterman, Allington and Sharp put forward a third suggestion (1972). They posulated that plasminogen activator in the circulation holds or adsorbs to the fibrin of a thrombus and circulating plasminogen is converted to plasmin within the thrombus. In contrast to the Sherry hypothesis, they propose that the activator is fixed in the thrombus and plasminogen circulates through it. Tissue plasminogen activator from pig heart certainly does have a strong affinity for fibrin. (Thorsen et al 1972) and human vascular plasminogen activator seems to behave similarly. Other plasminogen activators such as urokinase and streptokinase - plasminogen complex do not have specific affinity for fibrin although Chesterman et al (1972) did describe some adsorption of these activators to fibrin.

As must be apparent from the above the natural mechanism of thrombolysis in vivo is poorly understood. However, Wiman and Collen (1978b) have proposed a molecular model for physiological fibrinolysis which helps explain the restricted action of plasmin in vivo. They state that the system is regulated at two levels: localised plasminogen activation at the fibrin surface;

and sequestration of the formed plasmin from circulating antiplasmin.

Plasminogen and in particular lys-plasminogen is adsorbed to fibrin (Thorsen 1975; Rakoczi et al 1978) and in contrast to what was earlier believed (Moroi and Aoki 1977) α_2 antiplasmin does not influence this adsorption (Rakoczi et al 1978). Adsorption of plasminogen activator from the blood onto the fibrin results in activation of the adsorbed plasminogen.

The plasmin which is formed seems to be directed towards certain bonds of special importance for the liquefaction of fibrin and there is interaction between lysine binding sites in plasmin and sensitive bonds in fibrin.

One or more lysine binding sites participate in the binding of α_2 antiplasmin to plasmin and it has been suggested that the same lysine binding sites are involved in the binding of plasmin to fibrin (Wiman and Collen 1978b). This would effectively prevent α_2 antiplasmin from reacting with plasmin in the presence of fibrin. Plasmin released from digested fibrin, on the other hand, would be rapidly and irreversibly neutralised by circulating α_2 antiplasmin.

CONCLUSION.

The key component of the fibrinolytic system is the single chain glycoprotein, plasminogen which is present in plasma and in most tissues. Plasminogen is the proenzyme

from which the highly active serine protease plasmin is formed following limited proteolysis by a plasminogen activator. Plasmin has broad substrate specificity but in vivo is rendered relatively specific for fibrin by the nature of the interactions of plasminogen and plasmin with the other components of the fibrinolytic system.

REFERENCES.

ALBRECHTSEN O.K. (1957). The fibrinolytic activity of human tissue. British Journal of Haematology 3, 284.

ALI, S.Y. & LACK, C.H. (1965). Studies on the tissue activator of plasminogen. Distribution of activator and proteolytic activity in the subcellular fractions of rabbit kidney. Biochemical Journal, 96, 63.

ALKJAERSIG, N., FLETCHER, A.P. & SHERRY, S. (1959). The mechanism of clot dissolution by plasmin. Journal of Clinical Investigation, 38, 1086.

AMBRUS, C.M. & MARKUS, G. (1960). Plasmin-antiplasmin complex as a reservoir of fibrinolytic enzyme. American Journal of Physiology, 199, 491.

AOKI, N., & VON KAULLA, K.N. (1971). Dissimilarity of human vascular plasminogen activator and human urokinase. Journal of Laboratory and Clinical Medicine, 78, 354.

AOKI, N., MASAAKI, M., MATSUDA, M. & TACHIYA, K. (1977). The behaviour of α_2 plasmin inhibitor in fibrinolytic states. Journal of Clinical Investigation, 60: 361,

ARNESEN, H. & FAGERHOL, M.K., (1972) α_2 macroglobulin, α_1 antitrypsin, and antithrombin III in plasma and serum during fibrinolytic therapy with urokinase. Scandinavian Journal of Clinical and Laboratory Investigation, 29, 259.

ASTRUP, T., (1977). The physiology of fibrinolysis. In: Thrombosis and Urokinase Ed. by Paoletti R., Sherry S.P. New York, Academic Press.

ASTRUP, T., (1978). Fibrinolysis: an overview. In: Progress in Chemical Fibrinolysis and Thrombolysis Vol.III. p.1, Ed. by Davidson J.F., Rowan, R.M, Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

ASTRUP, T., & PERMIN, P.M., (1947). Fibrinolysis in the organism. Nature. 159, 681.

ASTRUP, T., & ROSA, A.T. (1974). A plasminogen proactivator-activator system in human blood effective in the absence of Hageman factor. Thrombosis Research. 4, 609.

BACHMANN, F., FLETCHER, A.P., ALKJAERSIG, N., & SHERRY, S. (1964). Partial purification and properties of the plasminogen activator from pig heart. Biochemistry, 3, 1758.

BACK, N., AMBRUS, J.L., & MINK, I.B. (1961). Distribution and fate of I^{131} labelled components of the fibrinolysin system. Circulation Research, 9, 1208.

BARLOW, G.H., & LAZER, L., (1972). Characterization of the plasminogen activator isolated from human embryo kidney cells: comparison with urokinase. Thrombosis Research 1, 201.

BARNHART, M.I., & RIDDEL, J.M. (1963). Cellular localisation of profibrinolysin (plasminogen). Blood, 21, 306.

BERNIK, M.B., WHITE, W.F., OLLER, E.P., & KWAAN, H.C. (1974). Immunologic identity of plasminogen activator in human urine, heart, blood vessels, and tissue culture. Journal of Laboratory and Clinical Medicine. 84, 546.

BINI, A., & COLLEN, D. (1978). Measurement of plasmin-antiplasmin complex in plasma. A comparison of latex agglutination, latex agglutination inhibition and haemagglutination inhibition tests. Thrombosis Research 12: 389.

BONNAR, J., McNICOL, G.P., & DOUGLAS, A.S., (1969). Fibrinolytic enzyme system and pregnancy. British Medical Journal, 3, 387.

BRAKMAN, P. (1967). A Standarized Fibrin Plate Method and a Fibrinolytic Assay of Plasminogen, Ph.D. Thesis, University of Amsterdam, Amsterdam, Scheltema and Holkema,

BRAKMAN, P., ALBRECHTSEN, O.K., & ASTRUP, T. (1966). A comparative study of coagulation and fibrinolysis in blood from normal men and women. British Journal of Haematology, 12, 74.

CHESTERMAN, C.N., ALLINGTON, M.J. & SHARP, A.A. (1972). Relationship of plasminogen activator to fibrin. Nature, (New Biology) 238, 15.

CHRISTENSEN, L.R. (1945). Streptococcal fibrinolysis: a proteolytic reaction due to a serum enzyme activated by streptococcal fibrinolysis. Journal of General Physiology, 28, 363.

CHRISTENSEN, U., & CLEMMENSEN, I. (1977). Kinetic properties of the primary inhibitor of plasmin from human plasma. Biochemical Journal. 163, 389.

CLAEYS, H., MOLLA, A., & VERSTRAETE, M. (1973). Conversion of NH_2-terminal glutamic acid to NH_2-terminal lysine human plasminogen by plasmin. Thrombosis Research, 3, 515.

CLAEYS, H., & VERMYLEN, J. (1974). Physicochemical and proenzyme properties of NH_2-terminal glutamic acid and NH_2-terminal lysine human plasminogen. Biochimica Biophysica Acta, 342, 351.

COCCHERI, S., De ROSA, V., CAVALLARONI, K., & POGGI, M. (1978). Activation of fibrinolysis by means of sulfated polysaccharides. Present status and perspectives, In Progress in Chemical Fibrinolysis and Thrombolysis, Ed. by Davidson, J.F., Rowan, R.M., Samama, M.M. & Desnoyers, P.C. Vol 3, p461, New York, Raven Press.

COLE, E.R., & BACHMANN, F. (1977). Purification and properties of a plasminogen activator from pig heart, Journal Biological Chemistry, 252, 3729.

COLLEN, D., (1976). Identification and some properties of a new fast-reacting plasmin inhibitor from human plasma. European Journal of Biochemistry. 69, 209,

COLLEN, D., ONG, E.B., & JOHNSON, A.J. (1972a): Native human plasminogen. Federation Proceedings, 31, No.2. 229. (Abstract).

COLLEN, D., TYTGAT, D., CLAEYS, H., & WALLEN, P. (1972b). Metabolism of plasminogen in healthy subjects : effect of tranexamic acid. Journal of Clinical Investigation, 51, 1310.

COLLEN, D., & DeCOCK, F. (1974). Emergence in plasma during activation of the coagulation or fibrinolytic systems of neoantigens associated with the complexes of thrombin or plasmin with their inhibitors. Thrombosis Research 5: 777.

COLLEN, D., & DeCOCK, F. (1975). A tanned red cell haemagglutination inhibition immunoassay (TRCHII) for the quantitative estimation of thrombin-antithrombin III and plasmin α_2 antiplasmin complexes in human plasma. Thrombosis Research 7: 235.

COLLEN, D., GRIG, E.F., & JOHNSON, A.J. (1975). Human plasminogen: In vitro and in vivo evidence for the biological integrity of NH_2-terminal glutamic acid plasminogen. Thrombosis Research 7, 515.

COLLEN, D., & DeMAEYER, L. (1975). Molecular biology of human plasminogen. 1. Physicochemical properties and microheterogeneity. Thrombosis et Diathesis Haemorrhagica 34, 396.

COLLEN, D., DeCOCK, F., CAMBIASO, L., & MASSON, P. (1977). A latex agglutination test for rapid quantitative estimation of the plamin-antiplasmin complex in human plasma. European Journal of Clinical Investigation 7: 21.

COLLEN, D., SEMERARO, N., TELESFORO, P., & VERSTRAETE, M. (1978). The inhibition of plasmin by antithrombin III-heparin complex. II. During thrombolytic therapy in man. British Journal of Haematology. 39, 101.

DASTRE, A. (1893). Action proteolytique du serum sanguin prealablement traité par le chloroforme. Archives de physiologie normale et pathologique, 5, 661.

DELEZENNE, C., & POZERSKI, E. (1903). Action du serum sanguin sur la gelatine en presence du chloroforme. Compte rendue des séances de la Societé de Biologie et de ses filiales 55, 327.

DENIS, P.S. (1838). Essai sur l'application de la chimie a l'étude physiologique du sang de l'homme et a l'étude physiopathologique, hygienique et therapeutique des maladies de cette humeur. Paris : Béchet.

DEN OTTOLANDER, G.J., LEIJNSE, B., & CREMER-ELFRINK, H.M. (1967). Plasmatic and platelet anti-plasmins and anti-activators, Thrombosis et Diathesis Haemorrhagica, 18, 404.

DEUTSCH, D.G., & MERTZ, E.T. (1970): Plasminogen : purification from human plasma by affinity chromatography. Science, 170, 1095.

de WITT, C.D., (1964). Investigations of the inhibitors of the fibrinolytic system. Thrombosis et Diathesis Haemorrhagica, 12, 105.

DENYS, J., & de MARBAIX, H. (1889). Les peptonisations provoquées par le Chloroforme. La cellule 5, 197.

EDY, J., COLLEN, D., & VERSTRAETE, M. (1978). Quantitation of the plasma protease inhibitor α_2 antiplasmin with the chromogenic substrate S-2251, In Progress in Chemical Fibrinolysis and Thrombolysis, Ed. by Davidson, J.F., Rowan, R.M., Samama, M.M. & Desnoyers, P.C. Vol.3, p315. New York, Raven Press.

EKERT, H., FRIEDLANDER, I., & HARDISTY, R.M. (1970). The role of platelets in fibrinolysis. Studies on the plasminogen activator and anti-plasmin activity of platelets, British Journal of Haematology. 18, 575.

FANTL, P. (1962). Plasminogen activity of plasma and serum. Science, 135, 787.

FEARNLEY, G.R. (1965). Fibrinolysis p.3, Edward Arnold, London.

FRIBERGER, P., KNÖS, M., GUSTAVSSON, S., AURELL, L., & CLAESON, G. (1978). Methods for determination of plasmin, antiplasmin and plasminogen by means of substrate S-2251. Haemostasis, 7, 138.

GAFFNEY, P.J., BRASHER, M., LORD, K., & KIRKWOOD, T.B.L. (1977). Activation of plasminogen as a feature in its assay. Haemostasis, 6, 72.

GALLIMORE, M.J. (1979). Inhibitors of plasminogen activation present in human plasma. In. The Physiological Inhibitors of Blood Coagulation and Fibrinolysis. Ed. by Collen D, Wiman, B, Verstraete, M. p199, Amsterdam. Elsevier.

GANROT, P.O. (1966). Determination of α_2 macroglobulin as trypsin-protein esterase. Clinica Chimica Acta 14: 493.

GANROT, P.O., & SCHERSTEN, B., (1967). Serum alpha-2-macroglobin concentration and its variation with age and sex, Clinica Chimica Acta. 15: 113.

GRANELLI-PIPERNO, A., VASSALLI, J.D., & REICH, E. (1978). Secretion of plasminogen activator by human polymorphonuclear leucocytes. Modulation by glucocorticoids and other effectors, Journal of Experimental Medicine. 146, 1603.

GREEN, J.R. (1887). Note on the action of sodium chloride in dissolving fibrin. Journal of Physiology, 8, 372.

HEDIN, S.G. (1904). On the presence of a proteolytic enzyme in the normal serum of the ox. Journal of Physiology, 30, 195.

HEDNER, U., & NILSSON, I.M. (1965). Determination of plasminogen in human plasma by a casein method. Thrombosis et Diathesis Haemorrhagica, 14, 545.

HEDNER, U., NILSSON, I., & ROBERTSON, B. (1966). Determination of plasminogen in clots and thrombi. Thrombosis et Diathesis Haemorrhagica, 16, 38.

HEDNER, U., & MARTINSSON, G. (1978). Inhibition of activated Hageman factor (Factor XIIa) by an inhibitor of plasminogen activation (PA inhibitor). Thrombosis Research, 12, 1015.

HEIMBURGER, M. (1971). Basic mechanism of action of streptokinase and urokinase. Thrombolytic therapy. (19th Annual Symposium on Blood), Wayne State University, Detroit. Thrombosis et Diathesis Haemorrhagica, Supplement 47, p21.

HIGHSMITH, R.F., & KLINE, D.L. (1971). Kidney-primary source of plasminogen after acute depletion in the cat. Science, 174, 141.

HIJIKATA, A., FUGIMOTO, K., KITAGUCHI, H., & OKAMOTO, S. (1974). Some properties of the tissue plasminogen activator from the pig heart. Thrombosis Research, 4, 731.

HOLMBERG, L., BLADH, B., & ASTEDT, B. (1976). Purification of urokinase by affinity chromatography. Biochimica Biophysica Acta, 445, 215.

HUNTER, J. (1794). A treatise on blood inflammation and gun-shot wounds, p26. Nicol, London,

IATRIDIS, S.G., & FERGUSON, J.H. (1961). Effect of surface and Hageman factor on the endogenous or spontaneous activation of the fibrinolytic system. Thrombosis et Diathesis Haemorrhagica, 6, 411.

JOHNSON, A.J., KLINE, D.L., & ALKJAERSIG, N. (1969). Assay methods and standard preparations for plasmin, plasminogen and urokinase in purified systems. Thrombosis et Diathesis Haemorrhagica. 21, 259.

JONES, J.M., CREETH, J.M., & KEKWICK, R.A. (1972). Thiol reduction of human α_2 macroglobulin. Biochemistry. 127, 187.

KAPLAN, M.H. (1944). Nature and role of lytic factor in hemolytic streptococcal fibrinolysis. Proceedings of the Society of Experimental Biology and Medicine. 57, 40.

KAPLAN, A.P., & AUSTEN, K.F.(1972). The fibrinolytic pathway of human plasma. Isolation and characterisation of the plasminogen proactivator. Journal of Experimental Medicine. 136, 378.

KAPLAN, A.P., MANDLE, R.Jr. YECIES, L.D. & MEIER, H.L. (1977). Hageman factor dependent fibrinolysis. Thrombosis and Haemostasis. 38, 60.(abstract).

KLUFT, C. (1977). An inventory of plasminogen activators in human plasma. Thrombosis and Haemostasis. 38, 134 (abstract).

KLUFT, C. (1978). Blood fibrinolysis proactivators and activators in human plasma. Thesis. Dutch Efficiency Bureau. Pijnacher.

KOK, P., & ASTRUP, T. (1969). Isolation and purification of a tissue plasminogen activator and its comparison with urokinase. Biochemistry. 8, 79.

KOPITAR, M., STEGNAR, M., ACCETTO, B., & LEBEZ, D. (1974). Isolation and characterisation of plasminogen activator from pig leucocytes. Thrombosis et Diathesis Haemorrhagica. 31, 72.

KUCINSKI, C.S., FLETCHER, A.P., & SHERRY, S. (1968). Effect of urokinase antiserum on plasminogen activators: demonstration of immunologic dissimilarity between plasma plasminogen activator and urokinase. Journal of Clinical Investigation, 47, 1238.

LACKNER, H., & JAVID, J.P. (1973). The clinical significance of the plasminogen level. American Journal of Clinical Pathology, 59, 175.

LAURELL, C.B. (1966). Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Analytical Biochemistry 15, 45.

LESUK, A., TERMINIELLO, L., & TRAVER, J.H. (1965). Crystalline human urokinase: some properties, Science, 147, 880.

LESUK, A., TERMINIELLO, L., TRAVER, J.H., & GEOFF, J.L. (1967). Biochemical and biophysical studies of human urokinase. Thrombosis et Diathesis Haemorrhagica. 18, 293.

LEWIS, J.H., & FERGUSON, J.H. (1950). Studies on a proteolytic enzyme system of the blood. II. Fibrinolysokinase activators for profibrinolysin. Journal of Clinical Investigation. 29, 1059.

LIU, T.H., & MERTZ, E.T. (1971): Studies on plasminogen. IX. Purification of human plasminogen from Cohn fraction III by affinity chromatography. Canadian Journal Biochemistry, 49, 1055.

McCALL, D.C., & KLINE, D.L. (1965). Mechanism of action and some properties of a tissue activator of plasminogen. Thrombosis et Diathesis Haemorrhagica. 14, 116.

McCONNELL, D.J. (1972). Inhibitors of kallikrein in human plasma. Journal of Clinical Investigation 51: 1611.

MacFARLANE, R.G., & PILLING, J. (1947). Fibrinolytic activity of normal urine. Nature. 159, 779.

McNICOL, G.P., BAIN, W.H., WALKER, F., RIFKIND, B.M., & DOUGLAS, A.S. (1965). Thrombolysis studied in an artifical circulation. Thrombi prepared in vitro in a Chandler's tube. Lancet, 1, 838.

MANCINI, G., CARBONARA, A.O., & HEREMANS, J.F. (1965). Immunological quantitation of antigens by single radial immunodiffusion, Immunochemistry, 2, 235.

MARSH, N.A. (1981). Fibrinolysis p.1 John Wiley & Sons, New York.

MILLER, L.L., & BALE, W.F. (1954). Synthesis of all plasma protein fractions except gamma globulins by the liver. Journal of Experimental Medicine, 99, 125.

MILSTONE, J.H. (1941). A factor in normal human blood which participates in streptococcal fibrinolysis. Journal of Immunology, 42, 109.

MORAWITZ, P. (1906). Uber einige postmortale Blutverandenungen. Beitrage zur Chemischen, Physiologie und Pathologie, 8, 1.

MOROI, M., & AOKI, N. (1976). Isolation and characterization of alpha$_2$-plasmin inhibitor in human plasma. A novel proteinase inhibitor which inhibits activator induced clot lysis. Journal of Biological Chemistry. 251, 5956.

MOROI, M., & AOKI, N. (1977). Inhibition of plasminogen binding to fibrin by α_2-plasmin inhibitor. Thrombosis Research, 10, 851,

MULLERTZ, S., (1974). Different molecular forms of plasminogen and plasmin produced by urokinase in human plasma and their relation to protease inhibitors and lysis of fibrinogen and fibrin. BiochemistryJournal, 143, 273.

MULLERTZ, S., & CLEMMENSEN, I. (1976). The primary inhibitor of plasmin in human plasma. Biochemistry Journal, 159, 545.

NAKAHARA, M., & CELANDER, D.R., (1968). Properties of microsomal activator of profibrinolysin found in bovine and porcine heart muscle. Thrombosis et Diathesis Haemorrhagica, 19, 483.

NIEWIAROWSKI, S., & PROU-WARTELLE, O. (1959). Role di facteur contact (facteur Hageman) dans la fibrinolyse. Thrombosis et Diathesis Haemorrhagica. 3, 593.

NILEHN, J.E., & GANROT, P.O. (1967). Plasmin, plasmin inhibitors and degradation products of fibrinogen in human serum during and after intravenous infusion of streptokinase. Scandinavian Journal of Clinical and Laboratory Investigation, 20, 113.

NOLF, P. (1908). Contribution a l'étude de la coagulation du sang. V. La fibrinolyse. Archives International de Physiologie, 6, 306.

OGAWA, N., YAMAMOTO, H., KATAMINE, T., & TOJIMA, H. (1975). Purification and some properties of urokinase. Thrombosis et Diathesis Haemorrhagica. 34, 194.

OGSTON, D. (1977). Biochemistry of naturally occurring plasminogen activators. In Haemostasis, Biochemistry, Physiology and Pathology. Ed. by Ogston, D. p.221. London, John Wiley & Sons.

OGSTON, D., OGSTON, C.M., & FULLERTON, H.W. (1966). The plasminogen content of thrombi. Thrombosis et Diathesis Haemorrhagica 15, 220.

OGSTON, D., OGSTON, C.M., RATNOFF, O.D., & FORBES, C.D. (1969). Studies on a complex mechanism for the activation of plasminogen by kaolin and chloroform: the participation of Hageman factor and additional cofactors, Journal of Clinical Investigation, 48, 1786.

OPIE, E.L., & BARKER, B.I. (1907). Leucoprotease and anti-leucoprotease of mammals and birds. Journal of Experimental Medicine, 9, 207.

OSHIBA, S., & ARIGA, T. (1975). Purification and characterization of bilokinase, a biliary plasminogen activator. Thrombosis et Diathesis Haemorrhagica, 34, 319.(Abstract).

PAINTER, R.H., & CHARLES, A.F. (1962). Characterisation of a soluble plasminogen activator from kidney cell cultures. American Journal of Physiology, 212, 1125.

PLOW, E.F., & EDGINGTON, T.F. (1975). An alternative pathway for fibrinolysis I. The cleavage of fibrinogen by leucocyte proteases at physiological pH. Journal of Clinical Investigation, 56, 30.

RABINER, S.F., GOLDFINE, I.D., HART, A., SUMMARIA, L., & ROBBINS, K.C. (1969). Radioimmunoassay of human plasminogen and plasmin. Journal of Laboratory and Clinical Medicine, 74, 265.

RAKOCZI, I., WIMAN, B., & COLLEN, D. (1978). On the biological significance of the specific interaction between fibrin, plasminogen and antiplasmin. Biochimica Biophysica Acta. 540, 295.

RATNOFF, O.D., & COLOPY, J.E. (1955). A familial haemorrhagic trait associated with deficiency of a clot promoting fraction of plasma. Journal of Clinical Investigation, 34, 602.

RATNOFF, O.D., & ROSENBLUM, J. (1958). Role of Hageman Factor in the initiation of clotting by glass. Evidence that glass frees Hageman factor from inhibition. American Journal of Medicine, 25, 160.

RATNOFF, O.D., PENSKY, J., OGSTON, D., & NAFF, G.B. (1969). The inhibition of plasmin, plasma kallikrein, plasma permeability factor and the C'_1r subcomponent of the first component of complement by serum C_1 esterase inhibitor. Journal of Experimental Medicine, 129, 315.

REICH, E. (1975). Plasminogen activator: secretion by neoplastic cells and macrophages, In: Proteases and Biological Control. Cold Spring Harbor Conference on Cell Proliferation, Vol.2, p333. Ed. by Reich, E., Rifkin, D.B. and Shaw, E. Cold Spring Harbor Laboratory, Cold Spring Harbour, New York,

RICKLI, E.E., & ZAUGG, H. (1970). Isolation and purification of highly enriched tissue plasminogen activator from pig heart. Thrombosis et Diathesis Haemorrhagica. 23, 64.

RICKLI, E.E., & OTAVSKY, W.I. (1973): Release of an N-terminal peptide from human plasminogen during activation with urokinase. Biochimica Biophysica Acta. 295, 381.

RICKLI, E.E., & OTAVSKY, W.I. (1975). A new method of isolation and some properties of heavy chains of human plasmin. European Journal of Biochemistry. 9, 441.

ROBBINS, K.C., SUMMARIA, L., ELWYN, D., & BARLOW, G.H. (1965): Further studies on the purification and characterization of human plasminogen and plasmin. Journal of Biological Chemistry. 240, 541.

ROBBINS, K.C., & SUMMARIA, L. (1970): Human plasminogen and plasmin. In: Methods in Enzymology. Proteolytic Enzymes. Vol. XIX. p184. Ed. by Pearlman, G.E., & Lorand, L., New York, Academic Press.

ROBBINS, K.C., BERNABE, P., ARZADON, L., & SUMMARIA, L. (1972): The primary structure of human plasminogen. 1. The NH_2-terminal sequence of human plasminogen and the S-carboxymethyl heavy (A) and light (B) chain derivatives of plasmin. Journal Biological Chemistry. 247, 6757.

ROSING, D.R., BRAKMAN, P., REDWOOD, D.R., GOLDSTEIN, R.E., & BEISER, G.D. (1970). Blood fibrinolytic activity in man. Diurnal variation and the response to varying intensities of exercise. Circulation Research. 27, 171.

SCHREIBER, A.D., & AUSTEN, K.I. (1974). Hageman factor independent fibrinolytic pathway. Clinical and Experimental Immunology, 17, 587.

SEMAR, J., SKOZA, L., & JOHNSON, A.J. (1969). Partial purification and properties of a plasminogen activator from human erythrocytes. Journal of Clinical Investigation, 48, 1777.

SEMERARO, N., COLUCCI, M., TELESFORO, P., & COLLEN, D. (1978). The inhibition of plasmin by antithrombin-heparin complex. 1. In human plasma in vitro, British Journal of Haematology, 39, 91.

SHERRY, S. (1968). Fibrinolysis. Annual Review of Medicine, 19, 247.

SJOHOLM, I., WIMAN, B., & WALLEN, P. (1973). Studies on the conformational changes of plasminogen induced during activation to plasmin and by 6 - amino hexanoic acid. European Journal of Biochemistry, 39, 471.

SOBEL, G.W., MOHLER, S.R., JONES, N.W., DOWDY, A.B.C., & GUEST, M.M. (1952). Urokinase: an activator of plasma profibrinolysin extracted from urine. American Journal of Physiology, 171, 768.

SOBERANO, M.E., ONG, E.B., JOHNSON, A.J., LEVY, M., & SCHOELLMANN, G. (1976). Purification and characterization of two forms of urokinase. Biochimica Biophysica Acta. 445, 763.

SORIA, J., SORIA, C., & SAMAMA, M. (1978). A plasminogen assay using a chromogenic synthetic substrate: results from clinical work and from studies of thrombolysis, in Progress in Chemical Fibrinolysis and Thrombolysis, Vol.3. p337. Ed. by Davidson, J.F., Rowan, R.M., Samama, M.M., & Desnoyers, P.C. New York, Raven Press.

SOTTRUP-JENSEN, L., CLAEYS, H., ZAJDEL, M., PETERSEN, T.E., & MAGNUSSON, S. (1978). The primary structure of human plasminogen: isolation of two lysine-binding fragments and one 'mini'-plasminogen (MW 38,000) by elastase catalysed-specific limited proteolysis. In: Progress in chemical fibrinolysis and thrombolysis. Vol.3, p191. Ed. by Davidson, J.F., Rowan, R.M., Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

STEAD, N., KAPLAN, A.P., & ROSENBERG, R.D. (1976). Inhibition of activated factor XII by antithrombin-heparin cofactor. Journal of Biological Chemistry, 251, 6481.

STORMORKEN, H. (1975). Relation of the fibrinolytic to other biological systems. Thrombosis et Diathesis Haemorrhagica, 34, 378.

SUMMARIA, L., HSEIH, B., & ROBBINS, K.C. (1967). The specific mechanism of activation of human plasminogen to plasmin. Journal of Biological Chemistry, 242, 4279.

SUMMARIA, L., ARZADON, L., BERNABE, P., & ROBBINS, K.C. (1972). Studies on the isolation of the multiple molecular forms of human plasminogen and plasmin by isoelectric focusing methods. Journal Biological Chemistry, 247, 4691.

SUMMARIA, L., ARZADON, L., BERNABE, P., & ROBBINS, K.C. (1975). The activation of plasminogen to plasmin by urokinase in the presence of the plasmin inhibitor trasylol. The preparation of plasmin with the same NH_2 terminal heavy (A) chain sequence as the parent zymogen. Journal of Biological Chemistry, 250, 3988.

TAGNON, H.J., & PALADE, G.E.(1950). Activation of proplasmin by a factor from mammalian tissue. Journal of Clinical Investigation. 29, 317.

TEGER-NILSSON, A.C., FRIBERGER, P., & GYZANDER, E.,(1977). Determination of a new rapid plasmin inhibitor in human blood by means of a plasmin specific tripeptide substrate, Scandinavian Journal of Clinical and Laboratory Investigation, 37, 403.

THORSEN, S. (1975). Differences in the binding to fibrin of native plasminogen and plasminogen modified by proteolytic degradation. Influence of omega amino acids. Biochimica Biophysica Acta 393, 55.

THORSEN, S., GLAS-GREENWALT, P., & ASTRUP, T. (1972). Difference in the binding to fibrin of urokinase and tissue plasminogen activation. Thrombosis et Diathesis Haemorrhagica, 28, 65.

TILLETT, W.S., & GARNER, R.L. (1933). The fibrinolytic activity of haemolytic streptococci. Journal of Experimental Medicine, 58, 485.

TODD, A.S. (1959). Histological localization of fibrinolysin activator. Journal of Pathology and Bacteriology, 78, 281.

TODD, A.S., & HARGREAVES, L.N.McF: (1975). Identification of plasminogen activator in tissue, In Progress in Chemical Fibrinolysis and Thrombolysis, Ed. by Davidson, J.F, Samama, M.M. & Desnoyers, P.C. Vol.1. p217, New York, Raven Press.

TROLL, W., SHERRY, S., & NACHMAN, J. (1954). The action of plasmin on synthetic substrates. Journal of Biological Chemistry, 208, 85.

VERSTRAETE, M., VERMYLEN, J., & SCHETZ, J. (1978). Intermittent administration of streptokinase. Thrombosis and Haemostasis, 39, 61.

VIOLAND, B.M., & CASTELLINO, F.J. (1976). Mechanism of the urokinase catalyzed activation of human plasminogen. Journal of Biological Chemistry, 251, 3906.

WALLEN, P. (1978). Chemistry of plasminogen and plasminogen activation. In: Progress in Chemical Fibrinolysis and Thrombolysis, Vol.3. p167. Ed. by Davidson, J.F. Rowan, R.M. Samama M.M. & Desnoyers, P.C. New York, Raven Press.

WALLEN, P. (1980). Biochemistry of plasminogen. In: Fibrinolysis Ed. by Kline D.L. & Reddy K.N.N. p.1 Florida, CRC Press, Inc.

WALLEN. P., & WIMAN, B. (1970). Characterization of human plasminogen. I. On the relationship between different molecular forms of plasminogen demonstrated in plasma and found in purified preparations. Biochimica Biophysica Acta, 221, 20.

WALLEN, P., & WIMAN, B. (1972). Characterization of human plasminogen. II. Separation and partial characterization of different molecular forms of human plasminogen. Biochimica Biophysica Acta, 257, 122.

WALLEN, P., & WIMAN, B. (1975). On the generation of intermediate plasminogen and its significance for the activation. In: Proteases and Biological Control. Ed. by: Reid, E. Rifkin D.B. & Shaw E. p291. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York,

WALTHER, P.J., STEINMAN, H.M., HILL, R.L., & McKEE, P.A. (1974): Activation of human plasminogen by urokinase: partial characterization of a preactivation peptide. Journal Biological Chemistry, 249, 1173.

WARREN, B.A., & KHAN, S: (1974). The ultrastructure of the lysis of fibrin by endothelium in vitro. British Journal of Experimental Pathology, 55. 138.

WIMAN, B., & WALLEN, P. (1973). Activation of human plasminogen by an insoluble derivative of urokinase. Structural changes of plasminogen in the course of activation to plasmin and demonstration of a possible intermediate compound. European Journal of Biochemistry, 36, 25.

WIMAN, B., & WALLEN, P. (1975a). On the primary structure of human plasminogen and plasmin. Purification and characterization of cyanogen-bromide fragments. European Journal of Biochemistry. 57, 387.

WIMAN, B., & WALLEN, P. (1975b). Structural relationships between 'glutamic acid' and 'lysine' forms of human plasminogen and their interaction with the amino terminal activator peptide as studied by affinity chromatography. European Journal of Biochemistry, 50, 489.

WIMAN, B., & COLLEN, D. (1977). Purification and characterization of human antiplasmin, the fast-acting plasmin inhibitor in plasma. European Journal of Biochemistry, 78, 19.

WIMAN, B., & COLLEN, D. (1978a). On the kinetics of the reaction between human antiplasmin and plasmin. European Journal of Biochemistry, 84, 573.

WIMAN, B., & COLLEN, D. (1978b). Molecular mechanism of physiological fibrinolysis. Nature, 272, 549.

WOLOSOWICZ, N., & PROKOPOWICZ, J, (1970). Isolation and purification of antiplasmin from human granulocytes. Thrombosis et Diathesis Haemorrhagica. 24, 543.

YECIES I., MEIER, H., MANDLE, R.Jr. & KAPLAN, A.P. (1978). The Hageman factor dependent fibrinolysis pathway, In: Progress in Chemical Fibrinolysis and Thrombolysis, Ed. by Davidson, J.F. Rowan, R.M. Samama, M.M. & Desnoyers, P.C. Vol.3. p121. New York, Raven Press.

CHAPTER IV.

PLASMA FIBRINOLYTIC ACTIVITY - ITS MEASUREMENT AND VARIATIONS, NORMAL AND ABNORMAL.

INTRODUCTION.

Plasminogen, the inactive proenzyme of plasmin, is present in the blood and in practically all other body fluids. Activation of plasminogen can be achieved by intrinsic or extrinsic activator pathways.

At present the physiological and pathological significance of the intrinsic plasminogen activator pathways is not understood and measurement of their various components is beyond the scope of this thesis.

The extrinsic activator pathway has been studied by many different groups since the early 1930s. It is therefore better explored if not better understood.

Fibrinolytic activator is distributed widely throughout the tissues of the body. Activator can be detected in the endothelium of many blood vessels (Todd 1959; Todd 1964; Todd and Hargreaves 1975). It is not clear why the endothelium of some vessels is fibrinolytically active, but not that of others. The fibrinolytic activity of superficial veins varies considerably throughout the body. Arm veins have higher

activity than their corresponding leg veins (Pandolfi et al 1967). Fibrinolytic activity may vary even in different vessels in the same limb (Nicolaides et al 1976). Lack of activity may be due either to absence of plasminogen activator or to the co-existence of fibrinolytic inhibitors (Noordhoek-Hegt 1977).

Fibrinolytically active cells continuously release plasminogen activator into the fluids with which they are in contact. Hence the spontaneous (resting) fibrinolytic activity of the blood is due to activator released from the vascular endothelium. The differences observed in the activator content of the vessel walls are reflected in corresponding differences in the spontaneous fibrinolytic activity in the blood. Thus the resting fibrinolytic activity of blood collected from the arms is higher than that in blood drawn from the legs (Robertson et al 1972a).

The fibrinolytic activity of plasma is normally low and can be difficult to measure using the currently available methods. There is, however, a definite and measurable diurnal fluctuation in the levels observed in blood drawn from any one vessel (Rosing et al 1973) and any form of stress, physical or mental, normally results in increased plasma plasminogen activator activitity (Cash 1975a). Electroshock (Pina-Cabral and Rodrigues 1974), pneumoencephalography (Schneck and von Kaulla 1961) and injection of pyrogens stimulate an increase in plasma fibrinolytic activity.

Infusion of vasoactive drugs including adrenalin (Biggs et al 1947), salbutamol, isoprenaline (Gader et al

1973a), nicotinic acid (Robertson 1971) or vasopressin or its analogue 1-desamino-8-D-arginine vasopressin (DDAVP) (Gader et al 1973b; Mannucci et al 1975) result in a rapid transient increase in plasma fibrinolytic activity. Venous occlusion of a limb causes a marked local increase in blood activator levels (Clarke et al 1960; Nilsson and Robertson 1968).

The rise in plasma plasminogen activator in response to these stimuli has been attributed to increased release of activator from the endothelium. The mechanism by which this activator is released into the circulation however remains obscure.

Two main hypothesis have been put forward - adrenoceptor stimulation and a change in vascular motility.

Many of the above mentioned stimuli result in systemic release of catecholamine but the rise in fibrinolytic activity which follows the infusion of catecholamine can be only partly prevented by β adrenergic blockers (Cash et al 1970; Gader et al 1974). The adrenergic receptors involved in the release of plasminogen activators do not seem to be mediated by the known receptors and are not related to the β adrenergic receptors (Cash 1975b).

Other stimuli which produce increases in fibrinolytic activity, for example infusion of nicotinic acid, vasopressin or venous occlusion are unlikely to be associated with adrenoceptor stimulation. It has been suggested that a change in vessel calibre may be the

important factor determining the fibrinolytic response to these stimuli (Holemans 1963, Holemans et al 1967). However, many potent vasoactive drugs such as histamine and serotonin do not affect fibrinolytic activity. (Weiner et al 1959; Thomson et al 1964).

Studies on the fibrinolytic responses of isolated perfused organs have shown that they have quite a different pattern from that observed in vivo (Markwardt and Klocking 1976) and it has been suggested there may be a central control mechanism for activator release mediated through the hypothalamus (Cash 1978).

From the above it must be clear that any assessment of in vivo fibrinolytic activity should take account of not only the resting fibrinolytic activity of the blood but also the fibrinolytic response to stimuli normally expected to cause release of endothelial plasminogen activator.

MEASUREMENT OF FIBRINOLYTIC ACTIVITY.

Spontaneous Plasma Fibrinolytic Activity.

The fibrinolytic activity of blood is the net result of the balance between plasminogen activators and fibrinolytic inhibitors.

The physiological contribution of the intrinsic activator system is not clearly understood and specific measurement of its various components will not be discussed.

The presence and the fluctuating levels of extrinsic activator (vascular activator) in plasma have been reported using non-specific methods which, at best, measure mainly extrinsic activator, but also measure undefined amounts of intrinsic activator. Kluft (1978) has shown that it may be possible to define the proportion of the plasma plasminogen activation due to extrinsic activators by using C_1 inactivator to inhibit the contribution made by the intrinsic pathways. Whilst these methods have obvious attractions for research purposes their practical clinical use is yet to be established.

The most widely used methods for measuring plasminogen activator in plasma are all non-specific and affected not only by the activator content of the sample but the inhibitor content and in some methods by the levels of fibrinogen and plasminogen.

Plasminogen activator activity in normal plasma can be detected by spontaneous lysis of an incubated blood or plasma clot if the effect of the inhibitors is minimised by dilution or dilution and acidification. Preparation of a euglobulin fraction from the test sample further reduces the concentration of inhibitors and the plasminogen activator activity can be assayed using either a euglobulin clot lysis test or using a fibrin plate technique.

Whole Blood Clot Lysis Time.

The time taken for lysis to occur in a clot of whole blood incubated at 37°C is measured. While this test is

extremely simple and has been used as a screening test for hyperfibrinolysis it is insensitive and detects only severe hyperfibrinolysis.

Dilute Whole Blood Clot Lysis Time.(Fearnley et al 1957).

Blood is diluted to minimise the effect of the inhibitors. It is then clotted and the lysis time of the clot recorded. This test has been used to measure physiological variations in fibrinolytic activity and as a screening test for hyperfibrinolysis. Although the original method used phosphate buffer a modified test using acetate buffer has been described (Chohan et al 1975). The modified test gives a clearer end point and a shorter lysis time.

Plasma Clot Lysis Time.

Platelet poor plasma prepared from the blood sample is clotted and the lysis time measured. This test is slightly more sensitive to activators than the whole blood lysis test.

The three methods described above all give an indication of overall plasma fibrinolysis. They are all non-specific and are affected not only by the plasminogen activator content of the plasma but also by the levels of plasminogen, fibrinogen and inhibitors.

Isoelectric precipitation of the plasma precipitates plasminogen, fibrinogen and plasminogen activator whilst leaving most of the inhibitors in solution. Separation and resuspension of this precipitate allows plasminogen

activator activity to be assessed in a system largely devoid of fibrinolytic inhibitor. The only inhibitor present in significant quantity is C_1 inactivator (Kluft 1978).

Euglobulin Clot Lysis Time.

Many methods have been described for preparing the euglobulin fraction but they are basically all similar. Chilled, anticoagulated plasma is diluted and acidified either with carbon dioxide gas (Von Kaulla and Von Kaulla 1975) or with acetic acid (Kluft and Brakman 1975). The precipitated euglobulin fraction is then separated, resuspended in buffer and clotted with thrombin or calcium or both. The time taken for the clot to lyse is measured.

The final pH attained by acidification of the plasma is critical and probably optimally 5.9 as in the acetic acid method. Similarly a dilution of 1 in 10 is preferred since this is associated with less co-precipitation of C_1 inactivator than are greater dilutions.

The lysis time can be measured visually or more conveniently using an automated clot lysis recorder (Preston 1976). The euglobulin clot lysis time is affected by the fibrinogen and plasminogen content of the sample. Normally the time taken for lysis exceeds 150 minutes but in acute hyperfibrinolysis the lysis time may be less than 30 minutes.

Microeuglobulin Clot Lysis Time.

A microeuglobulin method has been described for use when it is possible to obtain capillary blood samples only. (Wsantapruek and Von Kaulla 1966).

Fibrin Plate Assay. (Kluft et al 1976).

Plasminogen activator activity can be assayed by measuring the amount of lysis produced by a standard volume of euglobulin fraction on a standardised plasminogen-rich fibrin plate. Time and care are required for the preparation of the fibrin plates and once the euglobulin fraction is applied to them, they must be incubated undisturbed at 37°C for 18 hours.

For these reasons this method is probably restricted to the research situation or for the investigation of chronic clinical problems. It has little place in the investigation of acute hyperfibrinolysis. In the research situation it is a useful method in that it is simple to modify the test by adding reagents to the euglobulin fraction as it is applied to the fibrin plate. In this way the effect of C_1 inactivator on the various activator pathways has been studied (Kluft 1978). The fibrin plate method is unaffected by the levels of plasminogen and fibrinogen in the test plasma. Factors affecting the fibrin plate assay are discussed in greater detail in Chapter V.

Radioactive Iodine - Fibrinogen Clot Method.

Plasminogen activator may be measured by following the release of radioactivity from a radioactive iodine - fibrinogen clot (^{125}I fibrinogen) (Hume 1964, Moroz and Gilmore 1975).

Chromogenic Substrate Assays of Plasminogen Activators.

An assay using the synthetic chromogenic substrate S 2251 (Kabi) has been described. (Latallo et al 1978).

Plasminogen Activator Release.

A variety of different stimuli have been used in an attempt to devise a method of assessing the individuals ability to release plasminogen activator.

Physical Exercise and Adrenaline.

Tests using physical exercise and tests using injections of adrenaline have been described (Cash and Allan 1967; Cash and Woodfield 1967; Cash et al 1970) but whilst these tests may have limited clinical applicability they are unsuitable for use in many patients.

Nicotinic Acid.

The injection of nicotinic acid has been used as a test of fibrinolytic responsiveness but the high percentage of non-responders in volunteers and the long refractory period which follows the injection make the use

of nicotinic acid unsuitable for general use. (Robertson 1971).

Venous Occlusion.

Localised venous occlusion of an arm for a standardised period of time has been shown to be a simple and safe means of stimulating the local release of plasminogen activator. Venous occlusion is produced by placing a sphygmomanometer cuff around the upper arm and inflating it to a pressure midway between diastolic and systolic. Originally 20 minutes venous occlusion was suggested by Robertson et al (1972b). However venous occlusion is painful and studies using shorter periods of 10 or 15 minutes have been described. The 5 minute venous occlusion test previously described by the author (Walker et al 1977) is useful only as a screening test since individuals appear to vary in the rate at which they respond to the stimulus of occlusion and very little response may be observed in the first 5 minutes in some normal individuals.

Vasopressin and DDAVP.

A close relationship has been demonstrated between the fibrinolytic response to vasopressin and the response to venous occlusion both in healthy volunteers and in patients with thrombotic disease (Aberg and Nilsson 1975). Vasopressin is, however, not suitable for use as an activator release stimulus in the clinical situation because it produces vasoconstriction and causes abdominal

pain. The synthetic derivative of vasopressin, DDAVP (1-desamino-8-D-arginine) enhances blood fibrinolytic activity to an even greater extent than does vasopressin itself. (Mannucci et al 1975). Since DDAVP has no vasoactive properties and no intestinal side-effects it has been used to evaluate plasminogen activator release in clinical studies.

Plasminogen Activator Content of Vessel Wall.

The histochemical film technique for demonstrating fibrinolytic activity was originally described by Todd in 1959 and later modified (Pandolfi et al 1972), to allow quantitation of the activity in the vessel wall. Frozen sections of the blood vessel are incubated in contact with plasminogen rich fibrin on a glass slide. The slide is then stained and the areas of lysis produced are scored according to their size and form. A major disadvantage of this test is that it requires the biopsy of a small vein and it is therefore of limited clinical applicability.

PHYSIOLOGICAL VARIATIONS IN FIBRINOLYTIC ACTIVITY.

Foetal Life and Infancy.

Fibrinolytic activity is present in foetal blood from an early stage of development. There have been conflicting reports on the amount of plasminogen activator normally detectable at birth. Some authors have reported

high levels of plasminogen activator, exceeding normal adult levels, in the blood of the neonate; these levels fall rapidly within the first few days of life (Grossman et al 1952; Phillips and Skrodelis 1958; Ekelund et al 1970). Others, however, have found only trace quantities of plasminogen activator in the blood of full-term babies. (Ambrus et al 1963; Ambrus et al 1965).

Plasminogen levels are low in the normal full-term infant (Markarian et al 1967; Quie and Wannamaker 1960) and may be undetectable in premature neonates (Ambrus et al 1963). However, plasminogen has been detected immunologically as early as 10 weeks gestation (Gitlin and Biasucci 1969). Levels of fibrinolytic inhibitors reported in the foetus and in the neonate have varied widely. Some authors have reported low levels of inhibitors in the neonate (Witt et al 1969; Phillips and Skrodelis 1958; Quie and Wannamaker 1960; Samartzis et al 1960) whilst others have reported normal adult levels. (Ambrus et al 1963; Beller et al 1966).

Age, Sex and Race.

The reported effect of age on fibrinolysis has varied. Some authors have found that plasminogen activator levels are unaffected by age (Sawyer et al 1960; Fearnley et al 1963; Hamilton et al 1974) but others have suggested that plasminogen activator levels may rise in old age (Hume 1961; Swan 1963; Robertson et al 1972c). Plasma plasminogen levels rise with age (Ogston and Ogston

1966) as do the inhibitor levels (Hamilton et al 1974).

Cash (1966) found spontaneous plasma fibrinolytic activity to be greater in women than in men but this was not confirmed by Hamilton et al (1974). The fibrinolytic response to venous occlusion is significantly greater in females over fifty years old than in similarly aged males (Robertson et al 1972c) but no significant differences were demonstrated when the fibrinolytic response was examined in younger subjects.

Fibrinolytic activity is higher in black African males than in white European males (Walker 1961; Ferguson et al 1970). Fibrinolytic activity is higher in people living at high altitudes (Chochan et al 1974). Plasminogen levels are higher in Bantus and East Africans than in Europeans (Barr et al 1973; Ferguson et al 1970; Lackner and Johnson 1967). Fibrinolytic inhibitors are higher in white people than in Nigerians and East Africans (Howell 1965; Barr et al 1973).

Diurnal Variation.

There is clear evidence of diurnal variation in plasma fibrinolytic activity with low levels being found in the early morning and peak levels between 5 and 8 pm (Fearnley et al 1957; Mann 1967; Rosing et al 1970; Menon et al 1967a). It appears that this diurnal variation is not entirely explained by physical activity (Fearnley et al 1957).

Arterio Venous Differences.

Fibrinolytic activity is lower in arterial than in venous blood. (Fearnley and Ferguson 1957; Naimi et al 1963). This has been ascribed to higher levels of plasminogen activator in the veins than in the arteries but Almer et al (1975) found no significant differences in the activator content of biopsy specimens of temporal arteries and superficial dorsal hand veins in patients with diabetes mellitus. There are no arterio venous differences in levels of plasminogen, fibrinogen or inhibitors (Ogston et al 1966).

Pregnancy.

Marked increases have been noted in the level of fibrinogen during pregnancy (Bonnar et al 1969; Talbert and Langdell 1964). Plasminogen levels also rise during pregnancy (Bonnar et al 1969). However, spontaneous plasma fibrinolytic activity decreases during pregnancy and may be barely detectable at term (Nilsson and Kullander 1967; Brakman 1966; Bonnar et al 1969; Bonnar et al 1970). Spontaneous plasma fibrinolytic activity remains very low during labour but rises rapidly after delivery of the placenta is complete.

The fibrinolytic response to exercise is decreased during pregnancy (Cash 1975a) and the response to venous occlusion decreases to become almost zero at term.

However, using the histochemical method of Pandolfi et al (1972), it has been shown that the plasminogen activator content of the vein walls decreases only slightly during pregnancy (Astedt et al 1970; Astedt 1972a; Astedt 1972b; Astedt 1972c) and Astedt has suggested the decrease in plasma fibrinolytic activity is due mainly to the effect of placental hormones inhibiting the release of activator from the vessel wall.

Exercise.

Biggs and her co-workers were the first to demonstrate that exercise significantly increases the overall plasma fibrinolytic activity (Biggs et al 1947). Many workers have subsequently confirmed this observation (Iatridis and Ferguson 1963; Cohen et al 1968; Hawkey et al 1975; Davis et al 1976; Cash 1966; Menon et al 1967b; Rosing et al 1970). The increase in fibrinolytic activity which results from exercise is shortlived and is not associated with a fall in fibrinogen or plasminogen.

The increase in fibrinolytic activity which follows exercise is similar to that seen in other stress situations or after the infusion of adrenalin. In contrast to the increase in factor VIII levels which follows exercise the increase in fibrinolytic activity cannot be prevented by β-adrenergic blocking agents (Britton et al 1976).

Mental Stress.

Emotional stress, for example the fear of surgery, increases overall plasma fibrinolytic activity (Ogston et al 1962; MacFarlane and Biggs 1946).

Smoking.

Opinions have differed on the effect of smoking on plasma fibrinolytic activity Pozner and Billimoria (1970) found that heavy smoking shortened the euglobulin lysis times but Dalderup et al (1970) reported reduced fibrinolysis in smokers.

Janzon and Nilsson (1975) examined the effect of smoking on resting plasma fibrinolytic activity and on the response to venous occlusion. They found no differences between smokers and non-smokers but found that smoking of six cigarettes shortly before blood sampling resulted in shortening of the euglobulin lysis time, presumably due to the combined effects of nicotine and carbon monoxide.

Alimentary Lipaemia.

Conflicting observations on the effect of alimentary lipaemia on fibrinolysis have appeared. Some groups have reported reduced plasma fibrinolytic activity after a fatty meal. (Billimoria et al 1959; Buckell and Elliot 1959) but others have failed to confirm this observation

(Ogston and Fullerton 1962; Hougie and Ayers 1960). Amongst those who have recorded an effect of alimentary lipaemia on fibrinolytic activity there is dispute about the effect of different types of fat. Some have observed inhibition of fibrinolysis after ingestion of animal fats only (Greig and Runde 1957; Gajewski 1961) whilst others have reported inhibition after the ingestion of both animal and vegetable fats (Farquar et al 1961).

FIBRINOLYSIS IN VARIOUS PATHOLOGICAL CONDITIONS.

Fibrinolytic activity is altered in a variety of pathological conditions. In some there are observable alterations in fibrinolytic activity which appear to be directly associated with and resultant from the pathological state - for example the changes in fibrinolysis recorded during and after surgical operation. In other conditions, altered fibrinolysis has been observed, and it has been suggested that a relationship may exist between the altered fibrinolytic activity and the pathogenesis of the disorder - for example many workers have suggested that reduced fibrinolytic activity may be an important factor in the aetiology of degenerative vascular disease and of thrombotic disorders.

Obesity.

The spontaneous fibrinolytic activity of the blood tends to be low in obese subjects (Shaw and MacNaughton

1963; Ogston and McAndrew 1964; Bennett et al 1966; Warlow et al 1972). Obesity is also associated with a poor fibrinolytic response to exercise (Ogston and McAndrew 1964) and to venous occlusion (Almer 1975). The plasminogen activator content of vessel walls is significantly more often low in overweight subjects (Almer and Janzon 1975).

Surgical Trauma.

Spontaneous plasma fibrinolytic activity is raised during operation (Macfarlane 1937; Macfarlane and Biggs 1946; Andersson et al 1962) but falls immediately post-operatively and remains subnormal for 3-11 days thereafter (Griffiths et al 1977; Knight et al 1977). The vessel wall activator content and the response to venous occlusion are both significantly decreased on the third post-operative day (Nilsson et al 1980) - possibly as the result of "exhaustion" during surgery. It has been suggested that decreased fibrinolytic activity in the post-operative period may be an important factor in the aetiology of post-operative deep venous thrombosis (Knight et al 1977).

Some studies have demonstrated a greater and earlier reduction in fibrinolytic activity post surgery in patients who subsequently developed deep venous thrombosis than in those who did not (Mansfield 1972; Gordon-Smith et al 1974) but other authors have been unable to detect a significant difference (Becker 1972). Interestingly

Gallus et al (1973) demonstrated antiplasmin activity may be higher on the first post-operative day in patients who subsequently develop deep venous thrombosis.

Liver Disease.

Spontaneous plasma fibrinolytic activity is often increased in cirrhotic liver disease (De Nicola and Soardi 1958). It is believed that this increased activity is the result of impaired hepatic clearing of plasminogen activator. α_2 antiplasmin and plasminogen levels are reduced in cirrhotic disease (Brakman et al 1973/74; Teger-Nilsson et al 1978).

Thombotic Disorders and Degenerative Vascular Disease.

It has been postulated that normally in vivo there is equilibrium between the coagulation system, in which thrombin converts fibrinogen to fibrin, and the fibrinolytic system which is responsible for the dissolution of fibrin (Astrup 1958). The haemorrhagic tendency which results from impairment of coagulation activity or from hyperactivity of the fibrinolytic system is due to disturbance of the normal equilibrium such that fibrin dissolution exceeds fibrin formation. It seems reasonable to expect that increased coagulation activity or decreased fibrinolysis, by upsetting the balance in favour of fibrin deposition, may predispose to thrombus formation. However, possible relationships between

clinical thrombotic disease and disturbed blood coagulation or fibrinolysis are complex and have proven practically difficult to investigate.

Although more than a hundred years have elapsed since Virchow first suggested that three factors were important in the pathogenesis of thrombosis:

the vessel wall,

the blood within the vessel and

the characteristics of the blood flow,

the relative importance of each of these members of the triad in thrombus formation remains unclear. Thrombosis tends to occur where there is disease of the vessel wall but to some extent, the sites at which thrombi form are determined by the nature of the blood flow within the vessel, thrombus formation occurring most commonly where the flow is turbulent. How much disturbances in the nature of the blood itself contribute to thrombus formation has proven extremely difficult to establish. Even if disturbances in the normal coagulation : fibrinolysis equilibrium in favour of fibrin deposition do not directly result in thrombus formation it is attractive to postulate that they increase the tendency to thrombosis.

The differing structures of arterial and venous thrombi suggest that fibrinolysis must have a different contribution to make to the evolution of each. Venous thrombi have a small platelet head and a large fibrin tail, and it is simple to conceive that, in theory, reduced fibrinolytic activity may predispose to the

formation of this type of thrombus. Arterial thrombi are predominantly platelets but they also require the presence of fibrin for stability, and reduced fibrinolytic activity, at least theoretically, may predispose to arterial thrombosis.

Venous Thrombosis.

An association between venous thrombosis and decreased fibrinolytic activity has been sought. Many studies have shown that fibrinolytic activity is reduced in conditions associated with a high incidence of venous thrombosis, for example, malignant disease (Rennie and Ogston 1975) and cardiac failure (Rawles et al 1973). Following mycocardial infarction (Bennett et al 1967) and as already mentioned, following trauma and surgery a phase of reduced fibrinolytic activity is observed (Chakrabarti et al 1969; Rawles et al 1975). Depressed fibrinolytic activity may be important in the aetiology of deep venous thrombosis in these conditions.

One of the earliest reports of disordered fibrinolysis in a patient with existing thrombosis was made by Nilsson et al (1961) when she observed a patient with widespread venous thrombosis associated with severely impaired fibrinolytic activity that seemed likely to be due to the presence of a potent activator inhibitor. Menon et al (1971) reported that blood fibrinolytic activity was reduced in patients with venous thrombosis when compared with age and sex-matched controls.

The fibrinolytic activity of vein walls has also been

found to be reduced in patients with deep venous thrombosis (Pandolfi et al 1969) and patients with recurrent venous thrombosis have lower fibrinolytic capacity and lower vein wall activator content than controls (Isacson and Nilsson 1972).

Studies on the relationship between defective fibrinolytic activity and the development of venous thrombosis certainly suggest an association but are in no way conclusive.

Arterial Disease.

The general term thrombotic arterial disease is used to cover a multitude of vastly differing disorders, from those disorders affecting major central arteries to those disorders affecting the smallest peripheral arterioles. The role of impaired fibrinolysis in the pathogenesis and natural history of such widely different diseases may be quite diverse.

As far as the group of conditions collectively labelled cutaneous vasculitis is concerned, a number of workers have reported impaired plasma fibrinolytic activity in a percentage of sufferers (Cunliffe 1968; Parish 1972; Cunliffe and Menon 1971). Many patients with idiopathic or scleroderma - associated Raynauds phenomenon have poor fibrinolytic activity (Cunliffe and Menon 1969).

In terms of arterial disease, however, the most important question to be answered is whether or not defective fibrinolysis is in any way implicated in the aetiology of atherosclerosis. The thrombogenic theory of

atherogenesis published by Von Rokitansky (1852) and reviewed by Duguid (1946, 1955) has been further extended by Astrup (1958) to suggest that depressed fibrinolysis, by allowing continuous deposition of fibrin, may predispose to atherosclerosis.

Because degenerative vascular disease is common in the Western world in middle and old age, it is certain that no study can ever reveal absolute differences between patient and control groups, since inevitably a high proportion of the control group must eventually also have occlusive vascular disease. However, several investigators have produced evidence that occlusive arterial disease is associated with reduced fibrinolytic activity. Nestel (1959) compared a group of 30 men with widespread occlusion of major leg arteries who presented with intermittent claudication with a group of 30 men of similar age with no symptoms of peripheral arterial disease. He found that the spontaneous fibrinolytic activity (as measured by the dilute blood clot lysis time) in the patient group was significantly lower than in the control group. Fearnley and Chakrabarti (1964) and Naimi et al (1963) have reported similar findings.

In an initial investigation of spontaneous plasma fibrinolytic activity in survivors of myocardial infarction and age-matched controls, (Chakrabarti et al (1966) found 45% of the patients under 60 years had defective fibrinolysis compared with 8% of those over 60 years and 10% of the controls.

Because this initial study was based on single

estimations only, they repeated the work using three estimations of dilute blood clot lysis times in 107 male survivors of myocardial infarction and 90 healthy age-matched control with normal electrocardiograms (1968). In this study, 32% of the patients and 12% of the controls were found, irrespective of age, to have reduced fibrinolytic activity on at least two tests. Not all investigators however, have been able to demonstrate defective fibrinolysis in patients with coronary heart disease (Katz et al 1963; Merskey et al 1960).

Some workers, in an attempt to assess not only the spontaneous plasma fibrinolytic activity but also the functional capacity of what is after all a dynamic system, have studied the fibrinolytic response to stimulation. Rosing et al (1973) demonstrated reduced fibrinolysis activity in patients with coronary heart disease in samples taken with the patients in basal state in the early morning and found that these patients also had a reduced diurnal increase in fibrinolytic activity. Unfortunately, although the difference between patients and controls was clear-cut, it did not reach statistical significance. However, this finding of reduced response to internal physiologic stimuli encouraged other workers to examine the response to other, artificial, stimuli. Walker et al (1977) demonstrated that patients with angiographic evidence of coronary heart disease had a significantly reduced fibrinolytic response to 5 minutes venous occlusion of an arm, when compared with controls with angiographically normal coronary arteries. These

studies suggest, but certainly do not prove, that a relationship exists between defective fibrinolytic activity and arterial disease.

FIBRINOLYTIC ENHANCEMENT. DOES IT PREVENT THROMBOSIS?

Whether or not impaired fibrinolytic activity may be important in the development or progression of thrombotic disorders could be of more than just academic interest. The concept of pharmacologic enhancement of fibrinolysis as a means of clearing vascular thrombotic occlusions has many potential applications and has appealed to many investigators.

Currently, streptokinase and urokinase have a limited clinical use in the acute thrombotic situation. There may be a need for other less aggressive agents for use in prophylaxis or in the subacute or chronic disease state. The theoretical possibilities have been reviewed by Davidson and Walker (1979) but the number of fibrinolytic drugs actually available remains strictly limited.

Many compounds have been studied and found to have fibrinolytic and thrombolytic properties that are, unfortunately, only demonstrable in vitro (Von Kaulla 1975). Considerably fewer have been studied in vivo, and of those found to have fibrinolytic properties, the majority have this only as a transient phenomenon because resistance develops rapidly. Anabolic steroids are the major exception to this, and in certain clinical

situations, these have been shown to enhance fibrinolytic activity for up to 5 years (Walker and Davidson 1978). In other situations, the combination of an anabolic steroid with a diguanide achieved sustained enhancement of fibrinolysis (Fearnley et al 1967).

Venous Disease.

Deep venous thrombosis with subsequent pulmonary embolism must be the greatest single threat to post-operative recovery. The "fibrinolytic shutdown", which usually occurs post-operatively, can be prevented (Brown et al 1971) using fibrinolytic agents, but it is difficult to assess how important a contribution to the prevention of deep venous thrombosis this "normalizing" of the fibrinolytic activity may make.

In a double-blind study, Fossard et al (1974) administered a combination of phenformin (100 mg) and ethyloestrenol (8 mg) or a placebo daily for three weeks before and for one week after gynaecologic surgery. Although the patients on the active therapy demonstrated significant increases in fibrinolytic activity (by dilute blood clot lysis time) at the end of the three weeks prior to surgery, post-operatively there were no differences between the treated group and the placebo group. The frequency of deep venous thrombosis diagnosed by the radioactive fibrinogen test was the same in the two groups, but perhaps treatment was given for an insufficient length of time before surgery.

In 1975, Nilsson of Malmo reported on a large series of 75 patients, 60 of whom had recurrent idiopathic deep venous thrombosis, 10 recurrent superficial thrombophlebitis, and 5 retinal vein thrombosis, each treated with 100 mg phenformin and 8 mg ethyloestrenol daily for periods of three to forty eight months. Prior to treatment, all of these patients had low plasminogen activator content in the walls of their superficial veins, and most had a reduced plasma fibrinolytic response to venous occlusion. Treatment produced significant increases in both spontaneous plasma fibrinolytic activity and in the response to venous occlusion. Similarly significant increases in the plasminogen activator content of the vein walls were noted. Before entering the study, the patients had each had between 2 and 20 episodes of venous thrombosis, but during therapy, only 9 thrombotic episodes were recorded, and in 5 of these the vessel wall fibrinolytic activity was low at the time of recurrence. Thus, the frequency of thrombotic episodes was markedly decreased when the fibrinolytic activity was being kept "normal".

There is some evidence that fibrinolytic enhancement may improve the clinical condition of patients with Behçets disease (Cunliffe et al 1973; Cunliffe and Menon 1969).

Stanozolol has been shown to produce significant fibrinolytic enhancement and subjective improvement in patients with long-standing lipodermatosclerosis (Browse et al 1977).

Arterial Disease.

Studies on the clinical effect of fibrinolytic enhancement in arterial disease are much more difficult to assess.

In 1969 Menon et al reported that 9 out of 9 patients with cutaneous vasculitis treated in an open study with ethyloestrenol (2 mg QID) and phenformin (50 mg BD) had enhanced fibrinolysis as measured by the euglobulin lysis time whilst on treatment. Seven out of the 9 showed clinical improvement after 4-6 weeks treatment and, in these 7 patients, all manifestations of cutaneous vasculitis had disappeared at the end of 10 weeks treatment. In a further open study, Gillam et al (1974) reported clinical improvement in 5 patients with 'atrophie blanche' treated with phenformin and ethyloestrenol.

However, probably the best study so far reported on the effects of anabolic steroids in cutaneous vasculitis is that reported in preliminary form by Dodman et al (1973) and in a more complete form by Cunliffe et al (1975). Of the 25 patients participating in the study, 15 improved clinically when treated with phenformin and ethyloestrenol and 12 also responded clinically to phenformin and stanozolol. Fourteen of the 15 patients who showed clinical response were followed up for 2 years during which clinical improvement was maintained - in 7 patients even after cessation of drug therapy.

All 25 patients showed significant shortening of their euglobulin lysis times when they were taking anabolic steroids.

Anabolic steroid therapy produces clinical improvement in Raynaud's phenomenon. Probably the largest published study of anabolic steroid therapy in Raynaud's phenomenon is that reported by Jarrett et al (1978). In an open study, 20 patients were each given Stanozolol 5 mg BD for 3 months, taken off anabolic therapy for 3 months and then given a further 3 months Stanozolol.

The mean dilute blood clot-lysis time was significantly shortened during each 3 month period of therapy and the mean lysis area produced by the resuspended euglobulin fraction on fibrin plates increased significantly during the second 3 months of treatment. During each pulse of anabolic therapy, the mean plasma fibrinogen level fell significantly. Significant improvement in the mean hand blood flow was recorded during the first course of treatment and this improvement was sustained during the off-treatment interval of 3 months. A further increase in hand flow was recorded after the second 3 months of stanozolol. There were significant increases in mean palm temperature during anabolic therapy and the mean temperature of the index fingers also increased significantly. The mean grip strength which was measured before and after the second course of treatment showed highly significant improvement.

The question of what part, if any, fibrinolytic therapy has to play in the management of degenerative disease of major arteries is difficult and poorly explored. In 1970 Chakrabarti et al reported on an open study of 10 patients with occlusive vascular disease who had received continuous treatment with phenformin 50 mg BD and ethyloestrenol 4 mg BD for 2-3 years. During ethyloestrenol therapy all of the patients had reduced platelet adhesiveness, 7 had increased fibrinolytic activity, and most of them had lowered levels of plasma fibrinogen and serum cholesterol. When the ethyloestrenol was replaced by stanozolol 5 mg BD for 6 months, the platelet stickness returned to pre-anabolic steroid levels, but the fibrinolytic enhancement and the reduction in serum cholesterol levels were sustained almost unchanged and the plasma fibrinogen levels were further reduced. Chakrabarti and his colleagues felt it inappropriate to comment on any possible clinical benefit of the anabolic steroid therapy in their study. However, Bielawiec et al (1978) observed clinical improvement in the majority of 28 patients with obliterative arteriosclerosis given daily phenformin 100 mg and stanozolol 7.5 mg stating, without recording details, that their walking distances improved. During stanozolol treatment fibrinolytic activity was increased and plasma fibrinogen levels were lowered. Cholesterol, total lipid and β-lipoprotein levels were all reduced during therapy and in contrast to previous reports platelet adhesiveness was reduced.

If therapeutic assessment is difficult in peripheral arterial disease it has proven an almost insurmountable problem for many investigators examining the effects of countless different therapeutic possibilities in coronary heart disease (CHD). With this preface, therefore, it can be appreciated that there have been to date no really good studies of the clinical effect of fibrinolytic therapy in CHD.

In 1970, Menon et al reported that 10 post-myocardial-infarction patients, given phenformin 100 mg plus stanozolol 5 mg daily, demonstrated enhanced fibrinolytic activity during therapy. He made no comment on the clinical findings in these patients.

In a double blind study, Davidson et al (1972) examined 34 men post-myocardial infarction given either: phenformin 100 mg plus stanozolol 10 mg daily; or placebo plus stanozolol 10 mg daily; or matched placebo tablets, for a period of 12 months. Throughout the study, the groups on active treatment showed significant shortening of their euglobulin lysis time. As in other studies, plasma fibrinogen levels were reduced during anabolic therapy, but, in contrast to other studies, this was not sustained beyond 3 months and no significant effect on the cholesterol level was noted. No attempt was made to examine the clinical effect. In a further report on this study, 17 of the patients had their antithrombin III levels measured during stanozolol therapy and were found to have significantly increased levels compared to their baselines (Walker et al 1975). Nine of these patients

were followed up for 60 months on anabolic therapy and were found to have sustained fibrinolytic enhancement (Walker and Davidson 1978) but as in previous reports no clinical comment was felt appropriate.

Clinical comments on the value of anabolic steroids in CHD tend to be vague and non-specific. Bielawiec (1978) reported on 27 patients with CHD given daily phenformin plus stanozolol 7.5 mg. As in their study of patients with peripheral arterial disease, they reported enhanced fibrinolysis, lowered fibrinogen levels and lowered cholesterol, total lipid and β-lipoprotein levels but in addition they also state that improved electrocardiograms were found during anabolic therapy.

It seems likely that as with other agents of potential therapeutic value in CHD, only a large study involving many patients over several years will produce any real information on whether or not fibrinolytic therapy has any place in the management of CHD.

CONCLUSIONS.

In this chapter methods for assessing plasma fibrinolytic activity, both that which occurs spontaneously and that which results from a range of physiological and pharmacological stimuli, have been explored.

The levels of plasma fibrinolytic activity vary widely in normal healthy individuals according to their age, sex, race and habits.

A diversity of pathological conditions have been reported as being associated with altered fibrinolytic activity. In some instances, where fibrinolytic activity has been found to be reduced it has been suggested that the impaired ability to lyse fibrin may be causally important in the development of thrombosis. To date, however, there are no studies which demonstrate clearly that reduced fibrinolytic activity has caused either venous or arterial thrombotic disease.

Many investigators have speculated on the value of enhancing fibrinolytic activity in individuals with a thrombotic predisposition. Theoretically it is attractive to postulate that if individuals with impaired fibrinolytic activity could be identified, normalisation of their fibrinolytic system would be clinically beneficial. To date, however, there is only limited evidence available to support this concept.

REFERENCES.

ABERG, M. & NILSSON, I.M., (1975). Fibrinolytic response to venous occlusion and vasopressin in health and thrombotic disease. In Progress in Chemical Fibrinolysis and Thrombolysis, Vol.1. p121, Ed. by Davidson, J.F., Samama, M.M., & Desnoyers, P.C. New York, Raven Press.

ALMER, L.O. (1975). Effect of obesity on endogenous fibrinolytic activity in diabetes mellitus. Journal of Medicine, 6, 351.

ALMER, L.O. & JANZON L., (1975). Low vascular fibrinolytic activity in obesity. Thrombosis Research, 6. 171.

ALMER, L.O., PANDOLFI, M. & ABERG, M. (1975). The plasminogen activator activity of arteries and veins in diabetes mellitus. Thrombosis Research, 6, 177.

AMBRUS, C.M., WEINTRAUB, D.H., DUNPHY, D., DOWD, J.F., PAKREN, J.W., NISWANDER, K.R., & AMBRUS, J.I. (1963). Studies on hyaline membrane disease I. The fibrinolysin system in pathogenesis and therapy. Pediatrics, 32, 10.

AMBRUS, C.M., WEINTRAUB, D.H., NISWANDER, K.R., & AMBRUS, J.I. (1965). Studies on hyaline membrane disease II. The ontogeny of the fibrinolysin system. Pediatrics, 35, 91.

ANDERSSON, L., NILSSON, I.M., & OLOW, B. (1962) Fibrinolytic activity in man during surgery. Thrombosis et Diathesis Haemorrhagica, 7, 391.

ASTEDT, B., (1972a). Fibrinolytic activity during labour. Acta Obstetrica et Gynaecologica Scandinavica, 51, 171.

ASTEDT. B., (1972b). Fibrinolytic activity of veins in the puerperium. Acta Obstetrica et Gynaecologica Sandinavica, 51, 325.

ASTEDT,B., (1972c). Demonstration of significance of placenta in depression of fibrinolytic activity during pregnancy, Journal of Obstetrics and Gynaecology of the British Commonwealth, 79, 205.

ASTEDT, B., ISACSON, S., NILSSON, I.M., & PANDOLFI, M., (1970). Fibrinolytic activity of veins during pregnancy, Acta Obstetrica et Gynaecologica Scandanavica. 49, 171.

ASTRUP, T., (1958). The haemostatic balance. Thrombosis et Diathesis Haemorrhagica, 2, 347.

BARR, R.D., OUNA, N., & KENDALL, A.G. (1973). The blood coagulation and fibrinolytic enzyme systems in healthy adult Africans and Europeans - a comparative study. Scottish Medical Journal, 18, 93.

BECKER, J., (1972). Fibrinolytic activity of the blood and its relation to post-operative venous thrombosis of the lower limbs. A clinical study. Acta Chirurgica, Scandanavica. 138, 787.

BELLER, F.K., DOUGLAS, G.W., & EPSTEIN, M.D. (1966). The fibrinolytic enzyme system in the newborn. American Journal of Obstetrics and Gynaecology. 96, 977.

BENNETT, N., OGSTON, C., McANDREW, G., & OGSTON, D., (1966). Studies on the fibrinolytic enzyme system in obesity. Journal of Clinical Pathology, 19, 241.

BENNETT, N.B., OGSTON, C.M., & OGSTON, D. (1967). Studies on the blood fibrinolytic enzyme system following acute myocardial infarction. Clinical Science, 32, 27.

BIELAWIEC, M., MYSLIWIEC, M., & PERZANOWSKI, A., (1978). A combined therapy with phenformin plus stanozolol in patients with occlusive arterial disease and recurrent venous thrombosis. In: Progress in Chemical Fibrinolysis and Thrombolysis, Vol.3. p507, Ed. by Davidson, J.F., Rowan, R.M., Samama, M.M., & Desnoyers, P.C., New York, Raven Press.

BIGGS, R., MacFARLANE, R.G., & PILLING, J., (1947). Observations on fibrinolysis. Experimental activity produced by exercise or adrenaline. Lancet, 1, 402.

BILLIMORIA, J.D., DRYSDALE, J., JAMES, D.C.O., & MacLAGAN, N.F., (1959). Determination of fibrinolytic activity of whole blood with special reference to the effects of exercise and fat feeding. Lancet, 2, 471.

BONNAR, J., McNICOL, G.P., & DOUGLAS, A.S., (1969). Fibrinolytic enzyme system and pregnancy. British Medical Journal, 3, 387-389.

BONNAR, J., McNICOL, G.P., & DOUGLAS, A.S., (1970). Coagulation and fibrinolytic mechanisms during and after childbirth. British Medical Journal, 2, 200.

BRAKMAN, P., (1966). The fibrinolytic system in human blood during pregnancy. American Journal of Obstetrics and Gynaecology, 94, 14.

BRAKMAN, P., MOHLER, E.R., & ASTRUP, T., (1973/74). Blood fibrinolysis in liver disease. Haemostasis, 2, 209.

BRITTON, B.J., WOOD, W.G., SMITH, M., HAWKEY, C., & IRVING, M.H. (1976). The effect of beta adrenergic blockade upon exercise induced changes in blood coagulation and fibrinolysis. Thrombosis and Haemostasis. 35, 396.

BROWN, I.K., DOWNIE, R.J., HAGGART, B., MURRAY, G.H., ROBB, P.M. & SANTER, G.J. (1971). Pharmacological stimulation of fibrinolytic activity in the surgical patient. Lancet, 1, 774.

BROWSE, N.L., JARRETT, P.E.M., MORLAND, M. & BURNAND, K. (1977). Treatment of liposclerosis of the leg by fibrinolytic enhancement: A preliminary report. British Medical Journal, 2, 434.

BUCKELL, M., & ELLIOT, F.A. (1959). Effect of butter lipaemia on the rate of clot lysis in normal males. Lancet, 1, 662.

CASH, J.D., (1966). Effect of moderate exercise on the fibrinolytic system in normal young men and women. British Medical Journal, 2, 502.

CASH, J.D. (1975a). Physiological aspects of fibrinolysis. In: Synthetic Fibrinolytic Thrombolytic Agents, p5 Ed. by von Kaulla, K.N., Davidson, J.F., Springfield, Illinois, Charles C. Thomas.

CASH, J.D. (1975b). Neurohumoral pathways associated with the release of plasminogen activator in man. In: Progress in Chemical Fibrinolysis and Thrombolysis, Vol.1. p97, Ed. by Davidson, J.F., Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

CASH, J.D. (1978). Control mechanism of activator release. In: Progress in Chemical Fibrinolysis and Thrombolysis, Vol.3. p65. Ed. by Davidson J.F., Rowan, R.M., Samama, M.M., & Desnoyers, P.C., New York, Raven Press.

CASH, J.D., & ALLAN, A.G.E. (1967). Fibrinolytic response to moderate exercise and intravenous adrenaline in the same subjects, British Journal of Haematology, 13, 376.

CASH, J.D., & WOODFIELD, D.G., (1967). Fibrinolytic response to moderate, exhausitve and prolonged exercise in normal subjects. Nature (London), 215, 628.

CASH, J.D., WOODFIELD, D.G., & ALLAN, A.G.E. (1970). Adrenergic mechanisms in the systemic plasminogen activator response to adrenaline in man, British Journal of Haematology, 18, 487.

CHAKRABARTI, R., FEARNLEY, G.R., HOCKING, E.D., DELITHEOS, A. & CLARKE, G.M. (1966). Fibrinolytic activity related to age in survivors of myocardial infarction. Lancet, 1, 573.

CHAKRABARTI, R., HOCKING, E.D. FEARNLEY, G.R., MANN, R.O., ATTWELL, T.N. & JACKSON, D. (1968). Fibrinolytic activity and coronary heart disease. Lancet, 1, 987.

CHAKRABARTI, R., HOCKING, E.D., & FEARNLEY, G.R. (1969). Reaction pattern to three stresses electroplexy, surgery and myocardial infarction of fibrinolysis and plasma fibrinogen. Journal of Clinical Pathology, 22, 659.

CHAKRABARTI, R., EVANS, J.F., & FEARNLEY, G.R. (1970). Effects on platelet stickiness and fibrinolysis of phenformin combined with ehtyloestrenol or stanozolol. Lancet, 1, 591.

CHOHAN, I.S., SINGH, I., & BALAKRISHNAN, K. (1974). Fibrinolytic activity at high altitude and sodium acetate buffer. Thrombosis et Diathesis Haemorrhagica. 32, 65.

CHOHAN, I.S., VERMYLEN, J., SINGH, I., BALAKRISHNAN, K., & VERSTRAETE, M. (1975). Sodium acetate buffer - a diluent of choice in the clot cysis time technique. Thrombosis et Diathesis Haemorrhagica, 33, 226.

CLARKE, R.I., ORANDI, A., & CLIFFTON, F.F. (1960). Induction of fibrinolysis by venous occlusion. Angiology, 11, 367.

COHEN, R.J., EPSTEIN, S.E., COHEN, L.S., & DENNIS, L.H. (1968). Alterations of fibrinolysis and blood coagulation induced by exercise, and the role of beta-adrenergic-receptor stimulation. Lancet, 2, 1264.

CUNLIFFE, W.J. (1968). As association between cutaneous vasculitis and decreased blood fibrinolytic activity. Lancet, 1, 1126.

CUNLIFFE, W.J., & MENON, I.S. (1969). Treatment of Behçets syndrome with phenformin and ethyloestrenol. Lancet, 1, 1239.

CUNLIFFE, W.J., & MENON, I.S. (1971). The association between cutaneous vasculitis and decreased blood fibrinolytic activity. British Journal of Dermatology. 84, 99.

CUNLIFFE, W.J., ROBERTS, B.E., & DODMAN, B. (1973). Behcets syndrome and oral-fibrinolytic therapy. British Medical Journal. 2, 486.

CUNLIFFE, W.J., DODMAN, B., ROBERTS, B.E. & TEBBS, E.M. (1975). Clinicial and laboratory double blind investigation of fibrinolytic therapy of cutaneous vasculitis, In: Progress in Chemical Fibrinolysis and Thrombolysis Vol.1. p325. Ed. by Davidson, J.F. Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

DALDERUP, L.M., ZWARTZ, J.A., KELLER, G.H.M., SCHOUTEN, F., & VAN HAARD, W.B. (1970). Serum lipids, typing, fibrinolysis and smoking, British Medical Journal, 3, 323.

DAVIDSON, J.F., LOCHHEAD, M., McDONALD, G.A., & McNICOL, G.P. (1972). Fibrinolytic enhancement by Stanozolol: A double blind trial. British Journal of Haematology. 22, 543.

DAVIDSON, J.F., & WALKER, I.D., (1979). Synthetic fibrinolytic agents. Progress in Cardiovascular Diseases, Vol. XXI, 5, 375.

DAVIS, G.L., ABILDGAARD, C.F., BERNAYER, E.M., & BRITTON, M. (1976). Fibrinolytic and hemostatic changes during and after maximal exercise in males. Journal of Applied Physiology. 40, 287.

DeNICOLA, P., & SOARDI, F., (1958). Fibrinolysis in liver diseases. Study of 109 cases by means of the fibrin plate method. Thrombosis et Diathesis Haemorrhagica. 2, 290.

DODMAN, B., CUNLIFFE, W.J., ROBERTS, B.E., & SIBBALD, R. (1973). Clinical and laboratory double-blind investigation on effect of fibrinolytic therapy in patients with cutaneous vasculitis. British Medical Journal, 2, 82.

DUGUID, J.B. (1946). Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. Journal of Pathology and Bacteriology. 58, 207.

DUGUID, J.B. (1955). Mural thrombosis in arteries. British Medical Bulletin. 11, 36.

EKELUND, H., HEDNER, U., & NILSSON, I.M. (1970). Fibrinolysis in newborns. Acta Paediatrica Scandanavica. 59., 33.

FARQUHAR, J.W., MERIGAN, I.C., & SOKOLOW, M. (1961). Plasma fibrinolysis in man: the effect of chylomicrons derived from different dietary fats. Journal of Experimental Medicine, 113, 587.

FEARNLEY, G.R., & FERGUSON, J. (1957). Arteriovenous difference in natural fibrinolysis, Lancet, 2, 1040.

FEARNLEY, G.R., BALMFORTH, G., & FEARNLEY, E. (1957). Evidence of a diurnal fibrinolytic rhythm; with a simple method of measuring natural fibrinolysis. Clinical Science, 16, 645.

FEARNLEY, G.R., & CHAKRABARTI, R. (1964). Pharmacological enhancement of fibrinolytic activity of blood. Journal of Clinical Pathology. 17, 328.

FEARNLEY, G.R., CHAKRABARTI, R., & ALVIS, P.R.D. (1963). Blood fibrinolytic activity in diabetes mellitus and its bearing on ischaemic heart disease and obesity. British Medical Journal, 1, 921.

FEARNLEY, G.R., CHAKRABARTI, R., & HOCKING, E.D. (1967). Fibrinolytic effects of diguanides plus ethyloestrenol in occlusive vascular disease, Lancet. 2, 1008.

FERGUSON, J.C., MACKAY, N., & McNICOL, G.P. (1970). Effect of feeding fat on fibrinolysis, stypven time, and platelet aggregation in Africans, Asians and Europeans. Journal of Clinical Pathology. 23, 580.

FOSSARD, D.P., FIELD, E.S., KAKKAR, V.V., FRIEND, J.R., CORRIGAN, T.P. & FLUTE, P.T. (1974). Fibrinolytic activity and post-operative deep vein thrombosis. Lancet, 1, 9.

GADER, A.M.A., CLARKSON, A.R., & CASH, J.D.,(1973a). The plasminogen activator and coagulation Factor VIII response to adrenaline, noradrenaline, isoprenaline and salbutamol in man. Thrombosis Research, 2, 9.

GADER, A.M.A., Da COSTA, J., & CASH, J.D., (1973b). A new vasopressin analogue and fibrinolysis. Lancet, 2, 1417.

GADER, A.M.A., Da COSTA, J., & CASH, D.J., (1974). The effect of propranolol, alprenolol and practolol on the fibrinolytic and factor VIII response to adrenaline and salbutamol in man, Thrombosis Research, 4, 25.

GAJEWSKI, J., (1961). Effect of the ingestion of various fats on the fibrinolytic activity in normal subjects and patients with coronary heart disease. Journal of Atherosclerosis Research, 1, 222.

GALLUS, A.S., HIRSH, J., & GENT, M. (1973). Relevance of preoperative and post-operative blood tests to post-operative leg vein thrombosis. Lancet, 2, 805.

GILLAM, J.N., HERNDON, J.H., & PRYSTOWSKY, S.D. (1974). Fibrinolytic therapy for atrophie blanche. Archives of Dermatology, 109, 664.

GITLIN, D., & BIASUCCI, A., (1969). Development of γG, γA, γM, β1C/β1A. $C^1$1 esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, α_1 antitrypsin, orosomucoid, β lipoprotein, α_2 macroglobulin and prealbumin in the human conceptus. Journal of Clinical Investigation. 48, 1433.

GORDON-SMITH, I.C., HICKMAN, J.A., & Le QUESNE, L.P. (1974). Post-operative fibrinolytic activity and deep vein thrombosis. British Journal of Surgery. 61, 213.

GREIG, H.B.W., & RUNDE, I.A. (1957). Studies on the inhibition of fibrinolysis by lipids. Lancet. 2, 461.

GRIFFITHS, N.J., WOODFORD, M., & IRVING, M.H. (1977). Alteration in fibrinolytic capacity after operation. Lancet. 2, 635.

GROSSMAN, B.J., HEYN, R.M., & ROSENFELD, I.H., (1952). Coagulation studies in newborn infant: normal infants. Pediatrics. 9, 182.

HAMILTON, P.J., DAWSON, A.A., OGSTON, D., & DOUGLAS, A.S. (1974). The effects of age on the fibrinolytic enzyme system. Journal of Clinical Pathology. 27, 326.

HAWKEY, C.M., BRITTON, B.J., WOOD, W.G., PEELE, M., & IRVING, M.H. (1975). Changes in blood catecholamine levels and blood coagulation and fibrinolytic activity in response to graded exercise in man, British Journal of Haematology. 29, 377.

HOLEMANS, R. (1963). Increase in fibrinolytic activity by venous occlusion. Journal of Applied Physiology. 18, 1123.

HOLEMANS, R., MANN, L.S., & COPE, C., (1967). Fibrinolytic activity of renal venous and arterial blood. American Journal of Medicine and Science. 254, 330.

HOUGIE, C., & AYERS, F. (1960). Lipaemia and fibrinolytic potentiality. Lancet. 1, 186.

HOWELL, M.(1965). Comparison of fibrinolysis and coagulation in Nigerian and European men. Journal of Atherosclerosis Research. 5, 80.

HUME, R., (1961). The relationship to age and cerebral vascular accidents of fibrin and fibrinolytic activity. Journal of Clinical Pathology. 14, 167.

HUME, M. (1964). Radioactive (^{125}I) human plasma clots for assay of thrombolytic activity. Journal of Laboratory and Clinical Medicine. 63, 699.

IATRIDIS, S.G., & FERGUSON, J.H. (1963). Effect of physical exercise on blood clotting and fibrinolysis. Journal of Applied Physiology. 18,(Suppl.2). 337.

ISACSON, S., & NILSSON, I.M. (1972). Defective fibrinolysis in blood and vein walls in recurrent "idiopathic" venous thrombosis. Acta Chirurgica Scandanavia. 138, 313.

JANZON, L., & NILSSON, I.M. (1975). Smoking and fibrinolysis. Circulation. 51, 1120.

JARRETT, P.E.M., MORLAND, M., & BROWSE, N.L. (1978). Treatment of Raynau d's phenomenon by fibrinolytic enhancement. British Medical Journal. 2, 523.

KATZ, A.M., McDONALD, L., DAVIES, B. & EDGILL, M. (1963). Fibrinolysis and blood coagulation in ischaemic heart disease. Lancet. 1, 801.

KNIGHT, M.T.N., DAWSON, R., & MELROSE, D.G. (1977). Fibrinolytic response to surgery. Labile and stabile patterns and their relevance to post-operative deep venous thrombosis. Lancet. 2, 370.

KLUFT, C. (1978). Blood fibrinolysis. Proactivators and activators in human plasma. Thesis Dutch Efficiency Bureau, Pijnacker.

KLUFT, C., & BRAKMAN, P. (1975). Effect on euglobulin fibrinolysis: involvement of C_1-Inactivator. In: Progress in Chemical Fibrinolysis and Thrombolysis. Vol.I. p375, Ed. by Davidson, J.F., Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

KLUFT, C., BRAKMAN, P., & VELDHUYZEN-STOLK, E.C. (1976). Screening of fibrinolytic activity in plasma euglobulin fractions on the fibrin plate. In: Progress in Chemical Fibrinolysis and Thrombolysis Vol.II. p57 Ed. by Davidson, J.F., Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

LACKNER, H., & JOHNSON, A.I. (1967). The fibrinolytic system in South African white and Bantu men. Thrombosis et Diathesis Haemorrhagica. 18, 456.

LATALLO, Z.S., TEISSEYRE, E., & LOPACIUK, S. (1978). Assessment of plasma fibrinolytic system with use of chromogenic substrate. Haemostatis. 7, 150.

MacFARLANE, R.G. (1937). Fibrinolysis following operation. Lancet. 1, 10.

MacFARLANE, R.G., & BIGGS, R. (1946). Observations on fibrinolysis: spontaneous activity associated with surgical operation, trauma, etc., Lancet. 2, 862.

MANN, R.D., (1967). Effect of age, sex, and diurnal variation on the human fibrinolytic system. Journal of Clinical Pathology. 20, 223.

MANNUCCI, P.M., ABERG, M., NILSSON, I.M., & ROBERTSON, B. (1975). Mechanism of plasminogen activator and factor VIII increase after vasoactive drugs. British Journal of Haematology. 30, 81.

MANSFIELD, A.O. (1972). Alteration in fibrinolysis associated with surgery ad venous thrombosis. British Journal of Surgery. 59, 754.

MARKARIAN, M., GITHENS, J.H., JACKSON, J.J., BANNON, A.I., LANDLEY, A., ROSENBLUT, E., MARTORELL, R., & LUBCHENOC, I.O. (1967). Fibrinolytic activity in premature infants. Relationship of the enzyme system to the respiratory distress syndrome. American Journal of Diseases of Children. 113, 312.

MARKWARDT, F., & KLOCKING, H.P. (1976). Studies on the release of plasminogen activator. Thrombosis Research. 8, 217.

MENON, I.S., SMITH, P.A., WHITE, R.W.B., & DEWAR, H.A. (1967a). Diurnal variations of fibrinolytic activity and plasma 11-hydroxycoticosteroid levels. Lancet. 2, 531.

MENON, I.S., BURKE, F., & DEWAR, H.A. (1967b). Effect of strenuous and graded exercise on fibrinolytic activity. Lancet, 1, 700.

MENON, I.S., CUNLIFFE, W.J., & DEWAR, H.A. (1969). Preliminary report of beneficial effect of phenformin in combination with ethyloestrenol in treatment of cutaneous vasculitis and Behçet's syndrome. Postgraduate Medical Journal, May Supplement, 62.

MENON, I.S., CUNLIFFE, W.J., WEIGHTMANN, D., & DEWAR, H.A. (1970). Phenformin and stanozolol in blood fibrinolytic activity. British Medical Journal, 1, 428.

MENON, I.S., McCOLLUM, J.P.K., & GIBSON, A.L. (1971). Blood fibrinolytic activity in deep vein thrombosis. Lancet, 1, 242.

MERSKEY, C., GORDON, H., & LACKNER, H. (1960). Blood coagulation and fibrinolysis in relation to coronary heart disease. British Medical Journal, 1, 219.

MOROZ, L.A., & GILMORE, N.J. (1975). A rapid and sensitive ^{125}I-fibrin solid-phase fibrinolytic assay for plasmin. Blood, 46, 543.

NAIMI, S., GOLDSTEIN, R., & PROGER, S. (1963). Studies of coagulation and fibrinolysis of the arterial and venous blood in normal subjects and patients with atherosclerosis. Circulation, 27, 904.

NESTEL, P.J. (1959). Fibrinolytic activity of blood in intermittent claudication. Lancet, 2, 373.

NICOLAIDES, A.N., CLARK, C.T., THOMAS, R.O., & LEWIS, J.D. (1976). Fibrinolytic activator in the endothelium of the veins of the lower limb. British Journal of Surgery, 63, 881.

NILSSON, I.M. (1975). Phenformin and ethyloestrenol in recurrent venous thrombosis In Progress in Chemical Fibrinolysis and Thrombolysis, Vol.1. p1. Ed. by Davidson, J.F., Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

NILSSON, I.M., KROOK, H., STERNBY, N.H., SODEBERG, E., & SODERSTROM, M.N. (1961). Severe thrombotic disease in a young man with bone marrow and skeletal changes and witha high content of an inhibitor in the fibrinolytic system. Acta Medica Scandinavica. 169, 323.

NILSSON, I.M., & KULLANDER, S. (1967). Coagulation and fibrinolytic studies during pregnancy. Acta Obstetrica et Gynaecological Scandanavica. 47, 286.

NILSSON, I.M., & ROBERTSON, B. (1968). Effect of venous occlusion on coagulation and fibrinolytic components in normal subjects. Thrombosis et Diathesis Haemorrhagica. 20, 397.

NILSSON, I.M., HEDNER, U., & PANDOLFI, M. (1980). Physiology of fibrinolysis. In. Fibrinolysis Ed. by Kline, D.L., Reddy, K.N.N. p174. Boca Raton, Florida, C.R.C. Press.

NOORDHOEK-HEGT, V. (1977). Localization and distribution of fibrinolysis inhibition in the walls of human arteries and veins. Thrombosis Research, 10, 121.

OGSTON, D., & FULLERTON, H.W. (1962). Effect of alimentary lipaemia on plasma fibrinolytic activity. British Medical Journal. 2, 1288.

OGSTON, D., MacDONALD, G.A., & FULLERTON, H.W. (1962). The influence of anxiety in tests of blood coagulability and fibrinolytic activity. Lancet. 2, 521.

OGSTON, D., & McANDREW, G.M., (1964). Fibrinolysis in obesity. Lancet. 2, 1205.

OGSTON, C.M., & OGSTON, D. (1966). Plasma fibrinogen and plasminogen levels in health and in ischaemic heart disease. Journal of Clinical Pathology. 19, 352.

OGSTON, D., OGSTON, C.M., & BENNETT, B. (1966). Arteriovenous differences in the components of the fibrinolytic enzyme system. Thrombosis et Diathesis Haemorrhagica. 16, 32.

PANDOLFI, M., NILSSON, I.M., ROBERTSON, B., & ISACSON, S. (1967). Fibrinolytic activity of human veins. Lancet. 2, 127.

PANDOLFI, M., ISACSON, S., & NILSSON, I.M. (1969). Low fibrinolytic activity in the walls of veins in patients with thrombosis. Acta Medica Scandinavica. 186, 1.

PANDOLFI, M., BJERNSTAD, A., & NILSSON, I.M. (1972). Technical remarks on the microscopical demonstration of tissue plasminogen activator. Thrombosis et Diathesis Haemorrhagica. 27, 88.

PARISH, W.E. (1972). Cutaneous vasculitis: antigen-antibody complexes and prolonged fibrinolysis. Proceedings of the Royal Society of Medicine. 65, 276.

PHILIPS, L.L., & SKRODELIS, V. (1958). The fibrinolytic enzyme system in normal and haemorrhagic disease states. Journal of Clinical Investigation. 37, 965.

PRESTON, F.E. (1976). Automated euglobulin clot lysis time. In: Progress in Chemical Fibrinolysis and Thrombolysis Vol.II. p25. Ed. by Davidson, J.F., Samama, M.M., & Desnoyers, P.C. New York, Raven Press.

POZNER, H., & BILLIMORIA, J.D. (1970). Effect of smoking on blood-clotting and lipid and lipoprotein levels. Lancet. 1, 1318.

PINA-CABRAL, J.M., & RODRIGUES, C. (1974). Blood catecholamine levels, factor VIII and fibrinolysis after therapeutic electroshock. British Journal of Haematology. 27, 371.

QUIE, P.G., & WANNAMAKER, I.W. (1960). The plasminogen-plasmin system of newborn infants. Investigation of the nature and persistence of a deficiency of this system in human infants. American Journal of Diseases of Children. 100, 836.

RAWLES, J.M., OGSTON, D., & DOUGLAS, A.S. (1973). Studies on the blood fibrinolytic system in congestive cardiac failure. Clinical Science and Molecular Medicine. 45, 65.

RAWLES, J.M., WARLOW, C., & OGSTON, D. (1975). Fibrinolytic capacity of arm and leg veins after femoral shaft fracture and acute myocardial infarction. British Medical Journal. 2, 61.

RENNIE,J.A.N., & OGSTON, D. (1975). Fibrinolytic activity in malignant disease. Journal of Clinical Pathology. 28, 872.

ROBERTSON, B. (1971). Effect of nicotinic acid on fibrinolytic activity in health, in thrombotic disease and in liver cirrhosis. Acta Chirurgica Scandinavica. 137, 643.

ROBERTSON, B., PANDOLFI, M., & NILSSON, I.M. (1972a). Response of local fibrinolytic activity to venous occlusion of arms and legs in healthy volunteers. Acta Chirurgica Scandinavica. 138, 437.

ROBERTSON, B.R., PANDOLFI, M., & NILSSON, I.M. (1972b). "Fibrinolytic capacity" of healthy volunteers as estimated from effect of venous occlusion of arms. Acta Chirurgica Scandinavica. 138, 429.

ROBERTSON, B., PANDOLFI, M., & NILSSON, I.M. (1972c). "Fibrinolytic capacity" in healthy volunteers at different ages as studied by standardized venous occlusion of arms and legs. Acta Medica Scandinavica. 191, 199.

ROSING, D.R., BRAKMAN, P., REDWOOD, D.R., GOLDSTEIN, R.E., BEISER, G.D., & EPSTEIN, S.E. (1970). Blood fibrinolytic activity in man. Diurnal variation and the response to varying intensities of exercise. Circulation Research. 27, 171.

ROSING, D.R., REDWOOD, D.R., BRAKMAN, P., ASTRUP, T., & EPSTEIN, S.E. (1973). Impairment of the diurnal fibrinolytic response in man. Effects of ageing, type IV hyperlipoproteinaemia and coronary artery disease. Circulation Research, 32, 752.

SAMARTZIS, E.A., COOK, C.D., & RUDOLPH, A.J. (1960). Fibrinolytic activity in the serum of infants with and without the hyaline membrane syndrome. Acta Paediatrica. 49, 727.

SAWYER, D.W., FLETCHER, A.P., ALKJAERSIG, N., & SHERRY, S. (1960). Studies on the thrombolytic activity of human plasma. Journal of Clinical Investigation. 39, 426.

SCHNECK, S.A., & von KAULLA, K.N. (1961). Fibrinolysis and the nervous system. Neurology. 11, 959.

SHAW, D.A., & MacNAUGHTON, D. (1963). Relationship between blood fibrinolytic activity and body fatness. Lancet. 1, 352.

SWAN, H.T. (1963). Fibrinolysis related to age in men. British Journal of Haematology. 9, 311.

TALBERT, L., & LANGDELL, R.L. (1964). Normal values of certain factors in the blood clotting mechanism in pregnancy. American Journal of Obstetrics and Gynaecology. 90, 44.

TEGER-NILSSON, A.C., GYZANDER, E., HEDNER, U., MYRWOLD, H., NOPPA, H., OLSSON, R., & WALLMO, L. (1978). Antiplasmin and other natural inhibitors of fibrinolysis in clinical material. In Progress in Chemical Fibrinolysis and Thrombolysis. Vol.3 p327. Ed. by Davidson, J.F., Rowan, R.M., Samama, M.M., & Desnoyers, P.C. New York, Raven Press.

THOMSON, W.B., GREEN, J., & EVANS, I.L. (1964). The effect of some physiological stimuli on fibrinolytic activity in man measured by the heparin fractionation method. Journal of Clinical Pathology. 17, 341.

TODD, A.S. (1959). The histological localization of fibrinolysis activator. Journal of Pathology and Bacteriology. 78, 281.

TODD, A.S. (1964). Localisation of fibrinolytic activity in tissues. British Medical Bulletin. 20, 210.

TODD, A.S., & HARGREAVES, I.N.McF.(1975). Identification of plasminogen activator in tissue. In: Progress in Chemical Fibrinolysis and Thrombolysis. Vol.I. p217, Ed. by Davidson, J.F., Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

Von KAULLA, K.N. (1975). The synthetic approach to fibrinolysis thrombolysis. In: Synthetic Fibrinolytic Thrombolytic Agents. p53. Ed. by Von Kaulla, K.N., & Davidson, J.F., Springfield, Illinois, Charles C. Thomas.

Von KAULLA, K.N., & Von KAULLA, E. (1975). Remarks on the euglobulin lysis time. In: Progress in Chemical Fibrinolysis and Thrombolysis Vol.I. p131, Ed. by Davidson, J.F., Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

Von ROKITANSKY, C.A. (1852). A manual of pathological anatomy. Sydenham Society, London, 4, 261.

WALKER, W.R. (1961). Fibrinolytic activity of whole blood from South African Bantu and white subjects. American Journal of Nutrition. 9, 461.

WALKER, I.D. (1981). Anabolic steroid therapy in arterial disease. A Review. Scottish Medical Journal; S85.

WALKER, I.D., DAVIDSON, J.F., YOUNG, P., & CONKIE, J.A. (1975). Effect of anabolic steroids on plasma antithrombin III, α_2 macroglobulin and α_1 antitrypsin. Thrombosis et Diathesis Haemorrhagica. 34, 106.

WALKER, I.D., DAVIDSON, J.F., HUTTON, I., & LAWRIE, T.D.V. (1977). Disordered fibrinolytic potential in coronary heart disease. Thrombosis Research, 10, 509.

WALKER, I.D., & DAVIDSON, J.F.,(1978). Long-term fibrinolytic enhancement with anabolic steroid therapy. In: Progress in Chemical Fibrinolysis and Thrombolysis. Vol.3. p491. Ed. by Davidson, J.F., Rowan, R.M., Samama, M.M., & Desnoyers, P.C. New York, Raven Press.

WARLOW, C.P., McNEILL, A., OGSTON, D., & DOUGLAS, A.S. (1972). Platelet adhesiveness, coagulation and fibrinolytic activity in obesity. Journal of Clinical Pathology. 25, 484.

WASANTAPRUEK, S., & Von KAULLA, K.N. (1966). A serial microfibrinolysis test and its use in rats with liver bypass. Thrombosis et Diathesis Haemorrhagica, 15, 284.

WEINER, M., de CRINIS, K., REDISCH, W., & STEELE, M.J. (1959). Influence of some vasoactive drugs on fibrinolytic activity. Circulation. 19, 845.

WITT, I., MULLER, H., & KUNZER, W. (1969). Evidence for the existence of foetal fibrinogen. Thrombosis et Diathesis Haemorrhagica. 22, 101.

CHAPTER V.

METHODS USED FOR THE ASSESSMENT OF PLASMA FIBRINOLYTIC ACTIVITY.

INTRODUCTION.

In this chapter, are described the various standard procedures, clinical, laboratory and statistical which have been used in this study to determine plasma fibrinolytic activity both at rest and in response to the challenge of venous occlusion.

In addition a number of extra experiments on the effects of varying some of the procedures are described. These extra experiments were carried out by me personally during a visit to the Gaubius Institute, Leiden, Netherland and I would like to acknowledge the invaluable assistance and advice given to me by Dr. P. Brakman and Dr. C. Kluft during this visit.

STANDARD PROCEDURES.

BLOOD SAMPLES.

Collection.

Using a 21 swg needle, 9 ml of venous blood were collected into 1 ml of prechilled 31.3 g/l trisodium citrate in a polycarbonate tube. The tube was immediately replaced on melting ice.

Preparation of Platelet Poor Plasma.

Within 30 minutes of collection, the sample was centrifuged for 30 minutes at 3000 rpm at 4°C. 1.2 ml aliquots of the supernatant plasma were placed in 2 ml screw-capped "serum tubes", snap-frozen by dropping them into a flask of liquid nitrogen and then stored at -80°C.

Comment.

Plasminogen activator released during venous occlusion appears to be labile, its activity varying with pH and temperature (Nilsson et al 1970).

It has been claimed that freezing and subsequent thawing of specimens results in a serious loss of activator activity. Not all authorities, however, are convinced of the necessity to examine fresh specimens

(Kluft and Brakman 1975). In our experience, freezing does not significantly alter the fibrinolytic activity of euglobulin fractions (Walker et al 1976).

Providing contact with glass is avoided, the specimens are separated immediately after collection and the platelet poor plasma then frozen rapidly, there is no significant loss of activator activity with storage frozen for periods of up to three weeks. Practically it is obviously advantageous if, as in the studies reported here, specimens can be stored and examined in batches.

15 MINUTE VENOUS OCCLUSION TEST.

It is important that all variables should be considered and, as far as possible, a carefully standardised procedure adopted.

Describing the test procedure in advance, to the patient or control and explaining to him the reasons for carrying it out and necessity of standardising the procedure helps gain co-operation and hopefully lessens anxiety.

Test Procedure.

Subjects were instructed that they must fast from 9 pm the evening preceding testing, neither food nor fluids other than water being allowed. Smokers were asked to abstain from smoking during the period of fasting.

Testing was carried out at a standard time of day, in the morning at 9 am. Subjects were rested supine on a bed for at least 20 minutes prior to testing and throughout the test itself. It is helpful if the 20 minutes prior to testing are used to obtain details of the subjects name, age, etc., to obtain any important clinical information and generally to establish a relaxed rapport with the subject. During the period of venous occlusion the subject should not be left alone.

After 20 minutes rest, venous blood was collected from the right antecubital fossa without the use of a tourniquet. A sphygmomanometer cuff was then applied to the left upper arm and inflated to the pressure exactly midway between diastolic and systolic. A stop watch was started and the cuff was kept carefully inflated to the mid pressure for precisely 15 minutes. At the end of 15 minutes and immediately prior to deflating the cuff, venous blood was withdrawn from the left antecubital fossa.

Pressure was applied over the vein puncture sites until all bleeding was stopped, taking particular care with the puncture site on the occluded arm.

Comment.

The venous occlusion test is discussed in greater detail in the following Chapter (Chapter VI).

EUGLOBULIN FRACTIONS.

The term euglobulin fraction refers to the fraction obtained from diluted plasma by isoelectric precipitation at acid pH.

Materials.

Platelet Poor Plasma.

Prepared as previously described.

Acetic Acid.

Acetic acid 0.25 vol.% (0.44 M).

EDTA Buffer. (ionic strength 0.15, pH 7.75).

The EDTA buffer used contains,

0.05 M sodium diethylbarbiturate (Veronal),

0.10 M sodium chloride,

0.25% (W/V) gelatin,

and 0.079% (W/V) ethylenediamine tetra acetic acid (EDTA).

Since this buffer can be stored at 4°C, it is conveniently prepared in 1 litre volumes as follows:

Put 10.31 g of sodium diethylbarbiturate and 0.79g of EDTA in a flask with 740 ml of distilled water and 160 ml of 0.1 N hydrochloric acid. Add 2.5 g of gelatin and dissolve by warming gently. Adjust pH to 7.75 with 0.1 N hydrochloric acid and make up volume to 1 litre with distilled water.

Methods.

Immediately before fractionation, the frozen aliquot of plasma was thawed rapidly by placing it in a waterbath at 37°C. As soon as the plasma was thawed it was rapidly chilled by swirling it in a bath of iced water.

It is important that the preparation of the euglobulin fractions is carried out in the cold with, not only the plasma, but all of the reagents being chilled to the temperature of melting ice prior to starting and maintained at that temperature throughout the fractionation procedure. This is probably easiest done by placing the reagents and all the necessary tubes in racks standing in a large basin of melting ice.

9 ml of distilled water were pipetted into a siliconized glass tube (1.8 cm diameter x 9 cm long) and 1 ml of plasma was added. The tube containing the dilute plasma was transferred to a beaker of melting ice and the plasma stirred with a small magnetic stirrer, keeping the tube in melting ice. The pH of the solution was measured and then adjusted to pH 5.9 by adding 0.25% (V/V) acetic acid. It is important to stir gently during the titration and to bring down the pH slowly. The whole titration should take about 6-7 minutes.

After titration the solution must stand at least 15 minutes and no more than 120 minutes in ice before separating the preciptated proteins by centrifuging it for 10 minutes at 3000 rpm. at $\pm 4^{\circ}C$.

The supernate was discarded and the tube containing the euglobulin fraction was drained by inverting it on absorbent tissue for 3 minutes. The tube was then carefully dried inside using tissues and immediately replaced on ice. The "sticky" euglobulin fraction was broken up gently using a magnetic stirrer and then resuspended in 0.5 ml. EDTA buffer (200% euglobulin fraction).

The titration of the plasma pH down to pH 5.9 is most conveniently carried out using an automatic titrator (Automatic Titrator TTT 11, Radiometer, Copenhagen). The titrator electrode must be cleaned regularly and should be stored overnight in a room temperature solution of 0.1% (W/V) pepsin in 0.1 N hydrochloric acid. Before use, the electrode is rinsed with 0.1 N hydrochloric acid followed by distilled water and dried gently.

Comment.

Euglobulin fractionation of plasma is widely used in the study of plasma fibrinolytic activity because the euglobulin fraction contains considerably less inhibitors of the fibrinolytic system than does plasma. (Kluft 1976). A number of modifications for the preparation of euglobulin fractions have been described with various dilutions of the plasma and at various pH values (Cash 1966; Cucuianu 1966; Gallimore et al 1965). Kluft (1978) has shown that the highest fibrinolytic activity seems to

be obtained in fractions prepared at low ionic strength and at a pH of 5.9.

For reproducibility, the technique must be carefully standardised with regard to temperature, speed of stirring and rate of addition of acid. This is probably easiest achieved using an automatic titrator.

FIBRIN PLATES.

Materials.

Fibrinogen:

Dried fibrinogen powder was purchased from Poviet, Oss, Netherlands. Unless otherwise stated, the fibrinogen used throughout this study was plasminogen rich bovine fibrinogen (Batch No.106, Poviet, Oss, Netherlands).

Fibrin Plate Buffer: (ionic strength 0.15, pH 7.75).

The buffer used in the preparation of the fibrinogen solution was that described by Brakman (1967) containing,

0.050 M sodium diethylbarbiturate (Veronal)

0.093 M sodium chloride

1.66 mM calcium chloride

and 0.69 mM magnesium chloride

Since this buffer is stable at room temperature for about one week it is conveniently prepared in one litre volumes as follows:

Mix 10.31 g sodium diethylbarbiturate in a
flask with 600 ml distilled water,
250 ml 0.1N hydrochloric acid and
50 ml of salt solution (vide infra).

Adjust the pH with 0.1 N hydrochloric acid to 7.75 and then make up the volume to one litre with distilled water.

Salt solution :

for 1 litre, mix 4.89 g calcium chloride dehydrate
2.79 g magnesium chloride hexahydrate and
109.12 g sodium chloride.

Make up to one litre with distilled water.

Fibrinogen Solution.

Solid fibrinogen was dissolved in fibrin plate buffer to give a final concentration of 0.1% (W/V). Since it is important that the final ionic strength of the fibrinogen solution should be 0.15, account must be taken of the ions present in the fibrinogen starting material and the necessary adjustment in the ionic strength of the plate buffer made by adding distilled water. The fibrinogen solution was made, therefore, by mixing in a flask,

X mg of dry fibrinogen powder with V_B ml of fibrin plate buffer and adjusting the ionic strength with V_{H2O} ml of distilled water.

Where $X = (V_{sol}/F) \times 0.10 \times 100$

$V_{H2O} = (V_{sol} \times \mu'F)/0.15$

$V_B = Vsol - V_{H2O}$

When V_{sol} = Volume in ml of fibrinogen solution required

F = concentration % (W/V) of fibrinogen in the dry preparation

and $\mu'F$ = ionic strength of X mg of fibrinogen in Vsol ml of water.

Bovine plasminogen rich fibrinogen, Batch 106, Poviet, Oss, contains 80.7% protein of which 63% is clottable. To obtain 100 ml of 0.1% fibrinogen, (ionic strength 0.15), 159 mg of this fibrinogen (batch 106) was dissolved in 4 ml distilled water and 96 ml fibrin plate buffer (ionic strength 0.15). Immediately after addition of the solvent the mixture was stirred without foaming at room temperature for about 20 minutes.

Thrombin Solution.

Bovine thrombin, 5000 NIH units (Leo Pharmaceuticals, Copenhagan) was dissolved in 250 ml of saline containing 0.25% (W/V) gelatin to give a thrombin solution containing 20 NIH units/ml. This solution of thrombin was stored for no more than 24 hours at 4°C.

Methods.

Preparation of Fibrin Plates.

Six ml volumes of fibrinogen solution were pipetted into clean, flat based Petri dishes (90 x 15 mm - Sterilin) placed on a precisely levelled surface. 0.2 ml of thrombin solution was added to each and carefully and thoroughly mixed. The plates were then left for at least 30 minutes on the levelled table at room temperature to allow an even layer of fibrin to form.

Application of Test Solutions.

For measuring fibrinolytic activity in resuspended standard euglobulin fractions, 30 µl drops give measurable lysed zones. Since, for safety reasons, mouth pipetting with glass pipettes is forbidden in our laboratory, 30 µl drops were applied using an automatic micro-pipette (Clay Adams, USA). Each sample should be assayed at least in triplicate on three different fibrin plates.

Incubation of Fibrin Plates.

The fibrin plates, after application of the test solutions, were incubated for 18 hours in an incubator at 37°C. Care must be taken to ensure that the incubator shelves are exactly horizontal and that the incubator is situated where it will not be subjected to vibratory movements.

Measurement of Lysed Zones.

The diameters of the zones of lysis were measured using a simple device described by Haverkate (1972). Side lighting of the fibrin plates allows the lysed areas to be visualised easily and the diameters of the lysed zones are readily measured using a photographic negative of graph paper marked in millimetre squares placed under the plates. Two perpendicular diameters of each lysed zone were measured. For each sample the result was expressed as the mean diameter (in mm) of at least six diameters measured on at least three lysis zones.

Comment.

The fibrin plate method is a sensitive and accurate means of assessing fibrinolytic activity. Preparation of the plates however requires technical skill and meticulous standardisation of all steps of the procedure. The quality of the fibrinogen starting material is critical. Bovine plasminogen rich fibrinogen from Poviet has been found to be suitable for preparing fibrin plates.

DATA HANDLING.

Statistics.

The statistical methods used in the analysis of the data collected for this thesis have been limited to a very few fairly simple tests (Swinscow 1976).

a) Calculation of mean, variance, standard deviation and standard error

b) Student's t test to examine the differences between the means of a variable examined in two separate populations

c) Student's paired t test to examine the differences between the means of a variable in paired samples collected from a population

d) Calculation of correlation coefficients for two variables and the application of the t test to this correlation.

a) Mean, Variance, Standard Deviation, Standard Error.

Where n = total number of data points

Mean of x array $= \frac{\sum x}{n}$

$= \bar{x}$

Variance $= \frac{\sum (x-\bar{x})^2}{n-1}$

Standard deviation $= \sqrt{\frac{\sum (x-\bar{x})^2}{n-1}}$

$$\text{Standard error} = \frac{\text{Standard deviation}}{\sqrt{n}}$$

b) Student's t Test.

For each population, find the sum of the square of the differences from the mean value

i.e., $\sum (x_1 - \bar{x}_1)^2$ for population 1

and $\sum (x_2 - \bar{x}_2)^2$ for population 2

The square of the standard deviation (SD^2) of the two populations combined

$$= \frac{\sum (x_1 - \bar{x}_1)^2 + \sum (x_2 - \bar{x}_2)^2}{(n_1 - 1) + (n_2 - 1)}$$

The standard error of the difference between the means

$$= \sqrt{\frac{SD^2}{n_1} + \frac{SD^2}{n_2}}$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{SD^2}{n_1} + \frac{SD^2}{n_2}}}$$

Where n_1 = total number of data points in population 1

and n_2 = total number of data points in population 2

c) Student's Paired t Test.

Find the mean of the differences between each pair = $\bar{d}$.

Where n = the total number of pairs examined,

and SD^2 = the square of the standard deviation,

$$t = \bar{d} \div \sqrt{\frac{SD^2}{n}}$$

d) Correlation Coefficients.

The correlation coefficient, r, of two variables (x and y) is calculated,

$$r = \frac{\Sigma (x - \bar{x}) \quad (y - \bar{y})}{\sqrt{\Sigma (x - \bar{x})^2 \quad (y - \bar{y})^2}}$$

and $$t = r \sqrt{\frac{n - 2}{1 - r^2}}$$

where n = the number of data points for each variable.

Level of Significance.

Using single-tailed tests, P values of <0.05 are considered significant and P values of >0.05 not significant (NS).

Data Processing.

On completion of the study, the data were recorded and filed using an Apple II microcomputer with 48k of memory and associated 5 inch dual floppy disc drives (Apple Corporation, California). The programmes for filing the data and for its subsequent statistical analysis were custom designed for this study.

EXPERIMENTAL PROCEDURES.

DOSE RESPONSE CURVES FOR UROKINASE ON FIBRIN PLATES.

This experiment was designed to demonstrate two important facts.

1. The diameter of the lysed area produced by urokinase on fibrin plates is related to the concentration of the urokinase.

2. Gelatin added to the diluting buffer greatly enhances the observed lytic effect.

Materials.

Buffers.

a) Gelatin buffer was prepared as follows.

Put 10.31 g sodium diethylbarbiturate and 5.85 g sodium chloride in a flask with 700 ml distilled water
and 250 ml 0.1 N hydrochloric acid.
Add 2.5 g gelatin and warm gently to dissolve. Cool and adjust pH to 7.75 with 0.1 N hydrochloric acid. Make up to 1 litre with distilled water.

b) Gelatin-free buffer was prepared as follows.
Prepare exactly as for gelatin buffer but omitting the gelatin.

Urokinase (Leo, Copenhagan).

A stock solution of urokinase, 500 ploug units/ml in gelatin buffer was prepared.

Fibrin plates.

Fresh fibrin plates were prepared using bovine plasminogen-rich fibrinogen (as previously described).

Methods.

The following dilutions of urokinase were prepared from the stock solution,

a) in gelatin buffer, a 100% solution containing 25 ploug units/ml and 50%, 25%, 12.5%, 6.25% and 3.125% solutions containing equivalent amounts of urokinase.

b) in gelatin-free buffer, a 100% solution containing 25 ploug units/ml and 50%, 25%, 12.5%, 6.25% and 3.125% solutions containing equivalent amounts of urokinase.

Each of the dilutions was assayed six times by placing precisely 5 µl drops on fibrin plates. The plates were incubated for 18 hours at 37°C. Two perpendicular diameters of each lysed zone were measured and the result for each dilution was expressed as the mean of the twelve diameters recorded.

Results.

The mean diameter of lysis produced by each dilution is shown in Table 1.

Comment.

From these results, it is evident that the diameter of the lysed area produced by urokinase on fibrin plates is related to the concentration of the urokinase. Gelatin added to the diluting buffer, enhances the lytic effect of

TABLE 1.

Dilution of UK.	Diameter of lysis - mm. Gelatin.	Gelatin-free.
100%	23.7	22.1
50%	21.7	19.1
25%	18.9	14.4
12.5%	17.3	9.2
6.25%	14.6	4.8
3.125%	11.6	2.4

Diameter of lysis in mm produced by varying dilutions of urokinase in gelatin buffer and in gelatin-free buffer.

a given dilution of urokinase particularly where the urokinase is highly diluted. This enhancing effect of gelatin in the buffer is presumably the result of less urokinase being adsorbed onto the walls of pipettes and sample tubes in the presence of gelatin. The EDTA buffer used to resuspend the euglobulin fraction described in the standard procedure contains gelatin.

EFFECT OF VARYING SAMPLE HANDLING PROCEDURE.

This experiment was designed to demonstrate the importance of a rapid standardised sample handling procedure.

Blood Samples.

A sphygmomanometer cuff was applied to the right upper arm of a healthy female volunteer, inflated to a pressure of 105 mm Hg and maintained inflated for a period of 15 minutes. Two 9 ml samples of venous blood (A and B) were collected from this 'occluded' arm immediately prior to deflation of the cuff. Each 9 ml sample was immediately added to 1 ml prechilled 31.3 g/l trisodium citrate and mixed. Each sample was then subdivided into two 5 ml subsamples.

Sample Handling.

The four subsamples were handled as follows:

A_1 - Rapid at 0°C - This subsample was immediately spun at 3000 rpm, at 0°C for 30 minutes.

A_2 - Slow at 0°C - This subsample was placed in melting ice and left for 1 hour before spinning at 3000 rpm, at0°C for 30 minutes.

B_1 - Rapid at 0°C - This subsample was handled in exactly the same manner as A_1.

B_2 - Rapid room temperature - This subsample was immediately spun for 5 minutes at room temperature, 12,000 rpm using an Eppendorf high speed centrifuge 5412 (Eppendorf Geratebau, Hamburg).

After centrifugation, plasma was separated from each subsample and snap frozen in 0.6 ml aliquots.

Methods.

Fibrin Plates.

Plasminogen-rich bovine fibrin plates were prepared as previously described.

Euglobulin Fractionation.

Euglobulin fractions were prepared from each subsample by diluting the thawed plasma one part in ten with distilled water and adjusting the pH to 5.9 with

with distilled water and adjusting the pH to 5.9 with acetic acid (as already described). The euglobulin fractions were each resuspended in a volume of EDTA buffer equal to the starting volume of plasma - resulting in a 100% fraction.

Fibrin Plate Assay.

30 µl drops of each fraction were assayed in triplicate as already described.

Results.

The diameter of the lysed zones produced by euglobulin fractions prepared from each subsample is shown in Table 2. The very rapid room temperature handling produced a plasma with the greatest lytic activity (27.5 mm lysis). Delaying centrifugation for an hour resulted in a plasma with the least activity (24.4 mm lysis).

Comment.

It is evident from the above results, that measurable plasma fibrinolytic activity is altered by varying the sample handling procedure. Vascular plasminogen activator is labile. Plasma samples for measuring fibrinolytic activity are usually chilled and processed at 0-4°C. High speed centrifugation for 5 minutes at room temperature immediately after sample collection produced the plasma with the highest activity. Unfortunately this method is not suitable for routine use since the high speed

TABLE 2.

Sample	Diameter of lysis-mm
A_1 - rapid 0^oC	26.1
A_2 - slow 0^oC	24.4
B_1 - rapid 0^oC	26.0
B_2 - rapid RT	27.5

The diameter of lysis produced by samples of blood and handled at 0^oC and at room temperature (RT), rapidly and slowly - (after 1 hours delay).

centrifuge does not have the capacity for handling relatively large volumes of blood samples.

The above experiment demonstrates that even if plasma samples are handled using a meticulous cold technique a delay of as little as one hour from the time of collection until the time processing is commenced is associated with a measurable loss of fibrinolytic activity. It is essential that blood samples for fibrinolytic activity measurement are handled in a standardised fashion as quickly as possible after collection.

EFFECT OF VARYING pH OF EUGLOBULIN FRACTIONATION.

The effect of varying the pH of euglobulin fractionation on observed fibrinolytic activity was examined. Euglobulin fractions were prepared at pHs between 5.0 and 6.5 at constant ionic strength.

Materials.

Pooled Plasma:

A pool of platelet poor plasma was prepared from two healthy donors. Venous blood was collected from the antecubital fossa after 15 minutes venous occlusion with a sphygmomanometer cuff inflated to the pressure midway between diastolic and systolic pressures. Nine parts of venous blood were added to one part 31.3 g/l prechilled trisodium citrate and platelet poor plasma was prepared 'snap frozen' and stored as previously described.

Fibrin Plates:

Fresh fibrin plates were prepared as previously described using bovine, plasminogen-rich fibrinogen.

Acetic Acid:

Acetic acid 0.25 vol% (0.44 M).

EDTA Buffer:

Ionic strength μ = 0.15, pH 7.75, prepared as previously described.

Methods.

Four separate euglobulin fractions were prepared by diluting the plasma one part in ten in distilled water and then adjusting the pH of

one dilution to 5.0,
one dilution to 5.5,
one dilution to 5.9
and one dilution to 6.5

with 0.25% (V/V) acetic acid. The procedure for the separation of the euglobulin fractions was as previously described but the precipitated euglobulin fractions were each resuspended in volumes of EDTA buffer equal to the starting plasma volumes (100% euglobulin fractions). 30 μl drops of the resuspended precipitates were placed on fibrin plates and the plates incubated at 37°C for 18 hours. Each euglobulin fraction was assayed in triplicate and the fibrinolytic activity of each was expressed in

terms of the mean diameter of lysis which it produced.

Results.

The mean diameter of lysis produced by each fraction is shown in Table 3.

Comment.

As is obvious from Table 3 the fibrinolytic activity of the euglobulin fractions varies with differing pH and is maximal when the euglobulin fraction is precipitated at pH 5.9. This brief experiment is in keeping with meticulous studies carried out by Kluft (1978) on the effect of altering the pH of precipitation and also the effect of preparing euglobulin fractions at different ionic strengths. He found that maximal fibrinolytic activity was obtained in euglobulin fractions preciptated at low ionic strength with the pH adjusted to 5.9.

EFFECT ON OBSERVED FIBRINOLYTIC ACTIVITY OF VARYING THE FIBRINOGEN USED IN THE FIBRIN PLATES AND OF VARYING THE FINAL CONCENTRATION OF THE RESUSPENDED EUGLOBULIN FRACTION.

The apparent fibrinolytic activity of euglobulin fraction varies depending upon the fibrin substrate used to measure it.

TABLE 3.

	pH 5.0	pH 5.5	pH 5.9	pH 6.5
Diameter of lysis - mm.	20.7	21.4	23.7	22.4

Mean diameters of lysis produced by euglobulin fractions prepared at pH 5.0, pH 5.5, pH 5.9 and pH 6.5.

A euglobulin fraction was prepared and assayed on fibrin plates made from four different fibrinogen preparations.

During this experiment the opportunity was taken to assess also the effect of varying the volume of buffer used to resuspend the euglobulin precipitate.

Materials.

Pooled plasma:

A pool of platelet poor plasma was prepared from venous blood drawn from ten healthy donors. The pool was divided into 1.2 ml aliquots and stored as previously described.

Fibrinogen:

Fibrinogen for the preparation of the fibrin plates was obtained from the following sources:

(a) Plasminogen rich bovine fibrinogen prepared at the Gaubius Institute, Leiden, Netherlands.

(b) Plasminogen rich bovine fibrinogen. Poviet, Oss, Netherlands. (Batch No.114).

(c) Plasminogen poor bovine fibrinogen. Poviet, Oss, Netherlands. (Batch No.093).

(d) Plasminogen rich human fibrinogen. Kabi, Stockholm, Sweden.

Urokinase.

Urokinase (Leo, Copenhagen, Denmark) 10,000 ploug units was dissolved in buffer containing gelatin to give an end concentration of urokinase 20 ploug units/ml.

Buffers.

(a) Fibrin plate buffer.

(b) EDTA buffer for resuspending the euglobulin fraction.

(c) Gelatin buffer for diluting urokinase.

The preparation of these buffers was as previously described.

Thrombin.

Thrombin (Reagent Thrombin, Leo, Copenhagen, Denmark) 5,000 NIH units was dissolved in physiological saline containing 0.25% gelatin (W/V) to give a final concentration of thrombin 20 NIH units/ml.

Methods.

Preparation of fibrin plates:

A solution of each fibrinogen was prepared as previously described taking care to maintain the pH and the ionic strength the same for each solution. Using the method already described, a batch of fibrin plates was prepared from each fibrinogen solution.

Euglobulin fractions.

The euglobulin fraction was precipitated from the pooled normal plasma by diluting the plasma one part in ten with distilled water and adjusting the pH to 5.9 with acetic acid (as previously described).

The euglobulin fraction was resuspended in EDTA buffer equal to half the volume of the starting plasma. (200% euglobulin fraction). An aliquot of this 200% fraction was further diluted in buffer to produce a 100% euglobulin fraction. 30 µl. drops of each fraction were placed on each of the four types of fibrin plates, incubated at 37°C for 18 hours and the mean diameter of lysis produced by three drops was found for each fraction on each type of plate.

Urokinase.

5 µl drops of urokinase (20 ploug units/ml in gelatin buffer) were placed in triplicate on each of the four types of fibrin plate and the zones of lysis produced after 18 hours incubation at 37°C measured.

Results.

The mean diameters of lysis produced by the triplicate assays of the 100% euglobulin, the 200% euglobulin fraction and the urokinase solution on the fibrin plates made from each of the four different fibrinogen preparations are shown in Table 4.

Comments.

Firstly, it is obvious that there may be quite considerable differences in the apparent fibrinolytic activity of a solution depending upon the fibrin substrate used to measure it. As would be expected, urokinase did not lyse the Poviet plasminogen poor fibrin (Batch No.093) at all. Quite a marked difference was observed in the diameters of lysis produced by the urokinase on the different plasminogen rich fibrin plates, the human fibrin plates appearing to be less sensitive to urokinase than the bovine fibrin plates.

The euglobulin fraction contains significant amounts of plasminogen. The amount of plasminogen present in the fractions prepared would appear to have been sufficient to allow full expression of the fibrinolytic activity of these fractions even when the fibrin plates used contained very little plasminogen (Poviet, Batch No.093).

The sensitivity of fibrin plates varies according to the fibrinogen solution used in their preparation. This variable sensitivity is the result of many factors including the source of the fibrinogen and the amount of contaminants including plasminogen and fibrinolytic inhibitors.

It is important, that when a batch of fibrinogen preparation has been found to be suitably sensitive in the fibrin plate assay, sufficient quantities of this specific batch of preparation are available to complete a given study.

TABLE 4.

Diameter of lysis - mm	FIBRINOGEN PREPARATIONS Gaubius	Poviet 114	Kabi	Poviet 093
100% Euglobulin fraction.	8.9 mm	7.5 mm	8.6 mm	8.2 mm
200% Euglobulin fraction.	8.3 mm	7.8 mm	8.5 mm	8.8 mm
Urokinase.	23.1 mm	22.5 mm	18.3 mm	0

Mean diameters of lysis (mm) produced by 100% euglobulin fraction, 200% euglobulin fraction and Urokinase when applied to a variety of different substrate fibrins.

In the second place this experiment has demonstrated that doubling the concentration of the euglobulin fraction does not result in proportionally larger lysis zones. This is almost certainly because of the greater concentration of inhibitors present in the 200% fraction.

At this point it must be said that initial studies carried out in our laboratory, where Poviet fibrinogen is used, did suggest a slight advantage would be gained by preparing 200% euglobulin fractions. The experiment described above was carried out by me personally at the Gaubius Institute, Netherlands, after the bulk of the clinical material included in this thesis had been examined. I am now convinced that not only is there usually little to be gained by preparing 200% euglobulin fractions but because the precipitation of inhibitors in the euglobulin fraction is not quantitatively constant, 200% fractions are likely to compound an already complex situation.

INHIBITOR CONTENT OF EUGLOBULIN FRACTIONS.

Euglobulin fractionation of plasma is commonly used in the study of plasma fibrinolytic activity because the euglobulin fraction is said to be relatively free from fibrinolytic inhibitors (Kluft 1976).

The amounts of a number of antiproteases were measured in pooled resting plasma and in a 200% euglobulin fraction prepared from this pooled plasma.

Materials.

Pooled Plasma:

A pool of platelet-poor plasma was prepared as previously described from ten healthy donors. This pool was snap-frozen in 1.2 ml aliquots and stored at -80°C.

Euglobulin Fraction:

An aliquot of the pooled plasma was used to prepare a 200% euglobulin fraction (as previously described).

Inhibitor assays.

α_1 Antitrypsin (α_1AT), α_2 macroglobulin (α_2M), antithrombin III (ATIII), C_1 inactivator (C_1INA), interαtrypsin inhibitor (I α I) and α_1 antichymotrypsin (α_1X) levels were measured both in the pooled plasma and in the euglobulin fraction. The measurements were made immunologically by a single radial immunodiffusion technique using agar gel plates containing monospecific antisera (M partigen plates - Behringwerke AG)(Mancini et al 1965). Absolute values were calculated from calibration curves constructed by examining samples containing known dilutions of lyophilized protein standard plasma. (Behringwerke AG).

Results.

The amounts of each inhibitor present in the pooled plasma and in the euglobulin fraction are shown in Table 5.

TABLE 5.

	α_1AT mg%	α_2M mg%	ATIII mg%	C_1INA mg%	IαI mg%	α_1X mg%
Pooled Plasma	290	228	33	31	54	13
200% Euglobulin Fraction	0	0	0	17	51	13

Inhibitors present in plasma and in euglobulin fraction.

Comment.

A euglobulin fraction prepared from pooled plasma with normal levels of inhibitors contained immeasurable amounts of α_1 antitrypsin, α_2 macroglobulin and antithrombin III but compared with the pooled plasma, 55% C_1 inactivator, 94% interαtrypsin inhibitor and 100% α_1 antichymotrypsin. These results are similar to those recorded by Kluft (1976) but the percentage of the inhibitors detected was higher than he described (21% C_1INA, 52% IαI) because the euglobulin fraction we examined was a 200% fraction, whereas Kluft's was only a 100% fraction. Kluft has shown that the fibrinolytic activity of the euglobulin fraction is inhibited by the presence of C_1INA. The contribution of the other inhibitors to the inhibition of the fibrinolytic activity of the euglobulin fraction is not known but IαI is known to be a weak antiplasmin.

DISCUSSION.

Normally whole blood or plasma clots lyse very slowly. Euglobulin fractionation, by removing the bulk of the inhibitors which suppress plasma fibrinolytic activity, yields a fraction with considerable fibrinolytic activity. The fractionation process described here is based on methodology developed some years ago (Brakman et al 1966).

The standard method used for the preparation of euglobulin fractions for the work reported in this thesis

is acidification to pH 5.9 of a ten fold dilution of platelet poor plasma. Compared to other modifications of the euglobulin fractionation procedure, this method gives fractions with the highest fibrinolytic activity as measured on fibrin plates (Kluft 1978). This method produces fractions relatively low in inhibitor content but the inhibitors are not completely removed and they influence the apparent fibrinolytic activity of the fraction. α_2 macroglobulin, α_1 antitrypsin, antithrombin III and α_2 antiplasmin are present in the euglobulin fraction in trace amounts only. C_1 inactivator, inter α trypsin inhibitor and α_1 antichymotrypsin precipitate specifically but not quantitatively in the euglobulin fraction.

There is therefore probably no advantage to be gained by attempting to concentrate the fibrinolytic activity of the euglobulin fraction by resuspending it in a volume of EDTA buffer less than the starting plasma volume.

The plasminogen activators present in the euglobulin fraction are believed to be of both extrinsic (tissue or vascular activator) and intrinsic (Hageman factor dependent) origin (Kluft 1978). Not all the plasminogen activator activity from plasma is precipitated in the euglobulin fraction.

Since it was first described in 1947 (Permin 1947) the fibrin plate method has been widely used for measuring fibrinolytic activity. The fibrinolytic activity measured on plasminogen rich fibrin plates is mainly due to plasminogen activator. By using a single batch of

fibrinogen to prepare the fibrin plates, the amounts of fibrinogen and plasminogen in the plates is standardised. Although the fibrinolytically important proteins fibrinogen and plasminogen are partially precipitated in the euglobulin fractions, varying levels of these proteins in the euglobulin fraction do not significantly affect the assay.

The venous occlusion test described is based on observations made by Professor Nilsson's group working in Malmo, Sweden. The effect of venous occlusion on fibrinolytic activity and its acceptability as a clinical test will be discussed in Chapter VI.

REFERENCES.

BRAKMAN, P. (1967). Fibrinolysis, a standardised fibrin plate method and a fibrinolytic assay of plasminogen. Thesis. Amsterdam. (Scheltema and Holkema).

BRAKMAN, P., ALBRECHTSEN, O.K., & ASTRUP, T. (1966). A comparative study of coagulation and fibrinolysis in blood from normal men and women. British Journal of Haematology, 12, 74.

CASH, J.D., (1966). Effect of moderate exercise on the fibrinolytic system in normal young men and women. British Medical Journal. 2: 502.

CUCUIANU, M. (1966). Beta-lipoproteins and euglobulin lysis time. Thrombosis et Diathesis Haemorrhagica. 16, 687.

GALLIMORE, M.J., NULKAR, M.V., & SHAW, J.T.B. (1965). A comparative study of the inhibitors of fibrinolysis in human, dog and rabbit blood. Thrombosis et Diathesis Haemorrhagica. 14, 145.

HAVERKATE, F. (1972). A simple device for measuring diameters of fibrinolysis zones in fibrin plates. Haemostasis, 1, 55.

KLUFT, C. (1976). Occurrence of C_1 inactivation and other proteinase inhibitors in euglobulin fractions and their infuence on fibrinolytic activity. Haemostasis, 5, 136.

KLUFT, C. (1978). Blood fibrinolysis. Proactivators and activators in human plasma. (Thesis). Dutch Efficiency Bureau, Pijnacher.

KLUFT, C., & BRAKMAN, P. (1975). Effect of Flufenamate on euglobulin fibrinolysis: Involvement of C_1 inactivator. In: Progress in Chemical Fibrinolysis and Thrombolysis. Vol.1. p375. Ed. by Davidson, J.F., Samama, M.M. & Desnoyers, P.C. New York, Raven Press.

MANCINI, G., CARBONARA, A.O., & HEREMANS, J.F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2, 235.

NILSSON, I.M., PANDOLFI, M., & ROBERTSON, B.R. (1970). Properties of fibrinolytic activators appearing after venous stasis and intravenous injection of nicotinic acid. Coagulation, 3, (1), 13.

PERMIN, P.M. (1947). Properties of fibrinokinase-fibrinolysin system. Nature, (London). 160, 571.

SWINSCOW, T.D.V. (1976). Statistics at square one, London, British Medical Association.

WALKER, I.D., DAVIDSON, J.F., & HUTTON, I. (1976). "Fibrinolytic Potential". The response to a 5 minute venous occlusion test. Thrombosis Research, 8, 629.

CHAPTER VI.

SOME OBSERVATIONS ON THE VENOUS OCCLUSION TEST.

INTRODUCTION.

Good evidence exists to support the concept that an activator of fibrinolysis is formed in the blood vessel walls, in particular in the endothelium of the small veins. It is believed that this vascular activator of fibrinolysis is released into the circulation and that its presence accounts for the spontaneous fibrinolytic activity normally demonstrable in the blood.

There is also evidence that a low content of fibrinolytic activator in the vessel wall or impaired release of the activator into the blood stream predisposes to thrombosis (Isacson and Nilsson 1971).

Demonstration of an individuals ability to release plasminogen activator from the vessel wall may therefore be of clinical value. Spontaneous fibrinolytic activity is low and variations difficult to detect. Release of fibrinolytic activator is enhanced by a number of different stimuli including the injection of pyrogens, the injection of adrenaline, nicotinic acid or other vasoactive agents and physical or emotional stress

(Chapter IV). Physical exercise and the injection of adrenaline or nicotinic acid have been used clinically to assess fibrinolytic response (Amery et al 1965; Cash and Allen 1967) but, in the clinical situation they have limitations.

In 1960, Clarke et al demonstrated marked increases in local fibrinolytic activity following venous occlusion of a limb and since then this finding has been confirmed by many other workers (Amery et al 1962; Holemans 1963; Constantini et al 1969). It seems feasible that this increased fibrinolytic activity in response to venous occlusion would form the basis of a clinically useful test. Robertson (1971) developed a standardised method of measuring the "fibrinolytic capacity" using the response to 20 minutes venous occlusion. Unfortunately venous occlusion is painful when it is maintained for 20 minutes and several workers have therefore used shorter occlusion times. (Amery et al 1962; Iatridis et al 1966; Walker et al 1976). As shown by Åberg and Nilsson (1975), it is possible to identify poor responders with a shortened venous occlusion test but these shortened tests are probably less sensitive than full 20 minutes occlusion. It is the purpose of this Chapter to report on a number of aspects of the venous occlusion test as used in this thesis.

PATIENT ACCEPTABILITY OF THE VENOUS OCCLUSION TEST.

Blood Flow During Venous Occlusion.

In the resting relaxed arm the capillary and venous systems are not filled to capacity. When the upper arm is compressed at a pressure below systolic pressure the inflowing arterial blood is at first accommodated in this latent vascular space. As a result of the "buffering" effect of this latent space the pressure in the larger veins rises only gradually and may not surpass the compression pressure for about 5 minutes. Only when this stage is reached will venous blood start to leave the forearm again.

Increased venous and capillary pressure causes increased ultrafiltration and decreased blood flow. Even at compression pressures of 100 - 110 mm. mercury, Van Leeuwen (1964) demonstrated some venous flow persisted. He was able to show that decreasing blood flow during the compression levelled off after about 5 minutes but immediately after decompression there was a sudden short lasting "overshoot" which was followed by a longer period in which the blood flow was definitely lower than pre-occlusion.

Subjective Observations of Forearm Sensations.

The circulatory changes caused by venous compression produce pronounced effects on the appearances and sensations of the compressed arm. The observations recorded here are partly personal, subjective comments but are largely based on the comments of several hundred individuals who have been subjected to venous compression pressures of 100 - 120 mm. mercury.

During the first few minutes of compression the superficial veins visibly distend and an increasing cyanosis of the forearm develops. Often the skin develops a peculiar mottled appearance - red blotches appearing in the overall cyanosis. After 2 - 3 minutes the sensation of "tightness" under the occluding cuff is superseded by definite discomfort often most marked along the ulnar border. This discomfort increases and is usually maximal at about 5 - 7 minutes. It is often accompanied by itching of the skin. After about 10 minutes, the cyanosis does not appear to deepen further. About this time also sensations in the forearm gradually become dull and the arm feels "dead". No further increase in discomfort occurs until after about 15 - 20 minutes occlusion when paresis in the forearm begins to develop and on the two occasions on which we continued compression for 25 minutes, paralysis was noted in the wrist and hand muscles. (Similar observations were made by Van Leeuwen 1964). Immediately after sudden decompression the

superficial veins collapse, the skin returns to normal and for about 30 seconds the sensation in the forearm is best described as one of "relief". However, thereafter the skin may blanch and the fingers become white and cold. Subjectively this period, beginning about a minute after decompression and lasting 5 or 6 minutes, may be the most unpleasant of the whole test. After only 5 minutes compression, decompression produces little distress but after 15 minutes compression, release of the pressure results in marked paraesthesia. After 25 minutes occlusion, decompression is a singularly uncomfortable experience resulting in extreme and painful paraesthesia. Although the paralysis induced by the prolonged compression disappeared about 2 minutes after decompression, in one individual this was succeeded by Trousseau's sign, producing further distress.

A slight petechial rash is common after 15 minutes occlusion but fortunately marked rashes are not frequent. The tendency to develop purpura seems to be individual and is more common in females than males.

Venous thrombosis would appear to be a potential danger of this test but many authors have used venous occlusion tests of up to 20 minutes without reporting complications and we have performed many hundreds of 15 minute venous compression tests without ever observing signs of venous thrombosis.

Because of the increased incidence of paresis developing after about 20 minutes occlusion we decided that compression to this extent was unjustifiable for

clinical use in patients who are often already discomforted by serious vascular disease. We therefore limited our compression tests to a maximum of 15 minutes. Considering the sensations caused by even this period of occlusion the method is remarkably well tolerated - provided the patient is fully informed of the various sensations he will encounter and is not left alone during the period of compression.

THE EFFECT OF VENOUS OCCLUSION ON THE FULL BLOOD COUNT, PLASMA PROTEIN LEVELS AND FIBRINOLYTIC ACTIVITY.

In view of the amount of developmental work carried out by a number of authors on venous occlusion as a method of assessing fibrinolytic it was decided to limit investigation to the particular technique used in our own studies, namely, venous occlusion of the upper limbs at a pressure midway between diastolic and systolic and to examine the effects of a short period of occlusion - 5 minutes - and of a longer period of occlusion - 15 minutes.

The Venous Occlusion Tests.

Standardised 5 minute and 15 minute venous occlusion tests were developed. The 15 minute venous occlusion test is described in detail in Chapter V. The 5 minute venous occlusion test was performed in exactly the same manner with the exception that venous compression was maintained

for a period of 5 minutes instead of 15 minutes.

Subjects Studied.

The subjects studied were all healthy males aged 30-59 years. The criteria used to select these "controls" are detailed in Chapter VII.

Statistical Analysis.

The mean values and standard deviations for each set of results were calculated.

THE EFFECT OF LOCAL VENOUS OCCLUSION ON RED CELL COUNT, HAEMATOCRIT, WHITE CELL COUNT AND PLATELET COUNT.

Venous occlusion causes increased ultra-filtration through the vessel walls, with the result that there is increasing haemo-concentration depending on the compression pressure and on the length of time for which pressure is applied.

Blood Samples.

Blood taken, both from the resting arm and from the occluded arm was collected into Ethylenediamine tetra acetic acid (EDTA), 4 ml of blood being added to the dried powder in a standard "full blood count" tube (Staynes).

The samples were carefully mixed and tested the same day.

Laboratory Methods.

The red cells (RBCs) and the white cells (WBCs) were counted and the haematocrit calculated automatically using a Coulter S full blood counter (Coulter Electronics Ltd). Platelets were counted automatically using a Coulter thrombocounter (Coulter Electronis Ltd).

Results.

5 Minutes Venous Occlusion Test.

Five minute venous occlusion tests were carried out on a small number of healthy males. Each was tested on two occasions 72 hours apart. Full blood counts were not recorded on the second test - only haematocrit values. The mean levels of haematocrit, red cell count, white cell count and platelet count resting, after 5 minutes occlusion and the increment are shown in Table 6.

15 Minutes Venous Occlusion Tests.

Fifteen minute venous occlusion tests were carried out on healthy men aged 30-59 years. Whenever possible, subjects were tested twice with an interval of one month between Test 1 and Test 2. Platelet counts were carried out only at Test 1. The mean full blood count and platelet count results are shown in Table 7.

TABLE 6.

	No. of Subjects	RESTING $\bar{x}$	RESTING SD	OCCLUDED $\bar{x}$	OCCLUDED SD	INCREMENT $\bar{x}$	INCREMENT SD
RBCx10^{12}/L	32	4.9	0.07	5.5	0.14	0.57	0.24
HCT% 1	42	44.2	2.3	49.1	3.2	4.8	2.2
2	38	42.1	2.2	47.1	3.5	4.7	1.8
WBCx10^{9}/L	32	5.98	1.7	5.65	1.6	-0.36	0.41
PCx10^{9}/L	20	194.5	52.8	167.6	50	-25.7	24

The effect of 5 minutes venous occlusion on red cell count (RBC), haematocrit (HCT) white cell count (WBC) and platelet count (PC) in healthy males. The haematocrit is recorded for two separate tests (1 and 2).

TABLE 7.

	No. of subjects	RESTING x	RESTING SD	OCCLUDED x	OCCLUDED SD	INCREMENT x	INCREMENT SD
RBCx10^{12}/L							
1.	95	5.0	.39	6.1	.41	1.1	.28
2.	73	5.0	.32	6.1	.41	1.1	.25
HCT %							
1.	95	45.3	3.6	55.2	4.0	9.9	2.6
2.	73	45.0	2.8	54.2	4.3	9.3	3.2
WBCx10^{9}/L							
1.	95	6.55	1.6	6.78	1.6	0.21	.61
2.	73	6.14	1.8	6.27	1.8	0.14	.48
PCx10^{9}/L							
1.	55	221	64.2	207	60.3	-14	49.2

The effect of 15 minutes venous occlusion on red cell count (RBC) haematocrit (HCT), white cell count (WBC) and platelet count (PC) carried out on two occasions (1 and 2) in healthy males.

Comment.

Haemoconcentration occurs locally at the site of venous occlusion. After 5 minutes compression the mean RBC has risen by 0.6 x 10^2/L with an increase in mean haematocrit of 4.8%. Predictably 15 minutes occlusion is associated with even greater increases - the mean RBC increasing by 1.1 x 10^{12}/L and the mean haematocrit by 9.3 - 9.9%.

Interestingly the white cell count, unlike the red cell count does not appear to change significantly during venous occlusion either for 5 minutes or 15 minutes. Other workers have noted this phenomenon and have used it as evidence that leukocytes are not lost from the circulation (Holzknecht et al 1969). However, when this lack of change in the WBC count is compared with the considerable increase in the RBC count then there is evidence, if not of an absolute fall in the WBC count, at least of a relative fall. It is not necessary to postulate that this relative fall is due to complete loss of the WBCs from within the circulation or to total destruction of WBCs. White cells are effectively lost from the circulation if they are not in the main flow of blood sampled. It is possible, that during venous occlusion, WBCs are attracted towards the endothelial lining of the vessel either by chemotactic substances liberated from platelets adhering to an area of damage or by any one or number of a variety of chemotactic

substances related to the complement, kallikrein and fibrinolytic systems. Such WBCs adherent to the vessel wall will not be sampled and will appear to have been lost from the blood. If this process does occur then it is conceivable that lytic enzymes released from the adhering WBCs may contribute to the overall increase in fibrin lysing activity of post-occlusion blood samples.

The platelet counts like the white cell counts showed no significant change during venous occlusion. This also has been observed by other workers (Nilsson and Robertson 1968). As with the leukocytes, the failure of the platelet count to rise in accordance with the red cell count indicates a relative decrease. There are at least three possible explanations for this. Firstly, platelets may leave the vessel altogether - leaking through damaged vessel walls. This hypothesis is borne out in certain individuals who develop visible purpura. However, the results of plasma protein assays (which will be discussed later) indicate that "leakage" from the vessels of large molecules or particles is probably not usually significant. Secondly, even if large gaps in the vessel wall are not common, endothelial damage due to pressure or anoxia may result in platelets adhering to the vessel lining out of the stream sampled. Thirdly, Chamone and Vermylen (1977) have shown that 20 minutes venous occlusion results in the production of platelet aggregates visible in the post-occlusion samples. The automatic thrombocounter will either count an aggregate as a single platelet or not count it all, thus underestimating the

actual platelet count. Aggregating platelets release lytic enzymes which like those released from WBCs are capable of lysing fibrin.

EFFECT OF LOCAL VENOUS OCCLUSION ON ALBUMIN, FIBRINOGEN AND PLASMINOGEN LEVELS.

Blood Samples.

Blood taken both from the resting arm and from the occluded arm was collected into:

(a) Trisodium citrate - 9 ml. of blood were added to 1 ml. of pre-chilled 31.3 g/L trisodium citrate in a polycarbonate tube and immediately placed on melting ice.

(b) Glass tubes - 5 ml. of blood were allowed to clot in glass tubes.

Platelet poor plasma was separated from the citrated samples as described in Chapter V. Fibrinogen and plasminogen assays were performed on batches of samples which had been stored for no more than 1 month, the frozen aliquots being rapidly thawed by placing them in a water bath at 37°C. Serum was separated from the blood clotted in glass by spinning the clotted samples at 1,500 rpm - at room temperature - for 5 minutes. Aliquots of 1.2 ml of serum in plastic "serum" tubes were stored at -20°C.

Serum albumin assays were done on these samples after about 2 months storage.

Laboratory Methods.

Plasma Fibrinogen:

The plasma fibrinogen levels were measured by clotting diluted citrated plasma with excess thrombin (Dade fibrinogen determination kit) and measuring the clotting times with an automated mechanical clot timer. (Fibrosystem T.M.) Absolute values of fibrinogen were calculated from a calibration curve prepared by examining known dilutions (1:5; 1:15; 1:40) of a Fibrinogen Secondary Standard. (Dade Division).

Plasma Plasminogen:

Plasma plasminogen levels were assayed immunologically by a radial immunodiffusion technique using agar gel plates containing monospecific antiplasminogen antiserum (M Partigen plates - Behringwerke A.G.). Absolute values were calculated from calibration curves constructed by examining samples containing known dilutions of lyophilized protein standard plasma (Behringwerke, A.G.).

Serum Albumin:

Albumin in the serum samples was measured immunologically using goat antihuman-albumin serum in an automated immunoprecipitin system (Technicon - Autoanalyzer). Nephelometry was used to measure the formation of antigen - antibody complexes and the results

presented on a strip chart recorder as a series of peaks proportional to the concentration of albumin in the test serum.

Calculations.

Venous occlusion results in haemoconcentration. Since a constant amount of citrate solution was added to blood samples for plasma assays (1 ml. of citrate to 9 ml.of blood) the proportion of citrate was therefore somewhat higher in samples collected at the end of the occlusion period than in the resting samples. This results in an erroneously low result in all the post-occlusion plasma assays. To remove this error and to make the results from all the volunteers comparable, regardless of their haematocrit levels, each result, both resting and post-occlusion, was corrected to give "true" plasma values (i.e. as if there was no citrate present at all).

The correction applied was as follows,

$$\text{"True" value} = \text{Observed value} \times \frac{10 - \frac{\text{Hct} \times 9}{100}}{9 - \frac{\text{Hct} \times 9}{100}}$$

(where Hct = haematocrit).

Results.

The mean levels of serum albumin and plasma fibrinogen resting and after 5 minutes venous occlusion are shown in Table 8. The mean albumin level rose 0.4 g/L and the mean fibrinogen 0.5 g/L during 5 minutes occlusion. After 15 minutes venous occlusion (Table 9) the mean albumin had risen about 1.9 g/L, mean fibrinogen also about 1.9 g/L and the plasminogen about 6 mg/100 ml.

Comment.

Although small amounts of protein may leak or be catabolised, there is no evidence of significant loss of albumin, fibrinogen or plasminogen during venous occlusion.

Endothelial or vascular plasminogen activator has a molecular weight similar to that of albumin. Therefore, albumin seemed to provide a fairly good standard by which to assess whether or not the protein in which we were primarily interested, namely plasminogen activator, was likely to leak significantly through the vessel walls during occlusion. Although small amounts of albumin may be lost (Nilsson and Robertson 1968) we were unable to show that the loss was significant.

As with albumin, there was no evidence that either fibrinogen or plasminogen leaked through the vessel in large quantities nor was there evidence that large

quantities of fibrinogen or plasminogen were catabolised or activated - although it is appreciated that relatively vast quantities would need to be affected to produce a demonstrable fall in protein level during occlusion.

These observations on the effect of venous occlusion on protein levels provide indirect evidence to support the concept that the relative falls in white cell counts and platelet counts, already commented on, are unlikely to be completely explained by the loss of these cells out of the vessel.

THE EFFECT OF LOCAL VENOUS OCCLUSION ON PLASMA FIBRINOLYTIC ACTIVITY.

Blood Samples.

Blood was collected from the resting arm and from the occluded arm and platelet poor plasma prepared as described in Chapter V.

Laboratory Methods.

Euglobulin fractions were prepared and tested on plasminogen rich fibrin plates as described under the heading of standard methods (Chapter V.)

TABLE 8.

	No. of Subjects	RESTING $\bar{x}$	RESTING SD	OCCLUDED $\bar{x}$	OCCLUDED SD
ALBUMIN g/l	33	4.1	0.48	4.5	0.73
FIBRINOGEN g/l	33	2.6	0.48	3.1	0.53

The effect of 5 minutes venous occlusion on serum albumin and plasma fibrinogen levels is shown. The fibrinogen levels are corrected to "true" values and the means and standard deviations are shown for both albumin and fibrinogen, resting and post-occlusion.

TABLE 9.

	No. of Subjects.	RESTING $\bar{x}$	RESTING SD	OCCLUDED $\bar{x}$	OCCLUDED SD
ALBUMIN g/l	33	3.9	0.47	5.8	0.83
FIBRINOGEN g/l	33	2.4	0.42	3.3	0.57
PLASMINOGEN mg %	33	15.2	1.40	21.3	1.9

The effect of 15 minutes venous occlusion on serum albumin and plasma fibrinogen and plasminogen levels is shown. The fibrinogen and plasminogen levels are corrected to "true" values and the means and Standard deviations are shown for albumin, fibrinogen and plasminogen, resting and post-occlusion.

Results.

5 Minutes Venous Occlusion Tests.

Five minute venous occlusion tests were carried out on a number of healthy men.

Each was tested on two occasions 72 hours apart. The mean values of plasma fibrinolytic activity resting, after 5 minutes venous occlusion and the increment are shown in Table 10.

15 Minutes Venous Occlusion Tests.

Fifteen minute venous occlusion tests were carried out on a group of healthy males aged 30-59 years. Each subject was tested twice with an interval of one month between Test 1 and Test 2. The mean values of plasma fibrinolytic activity resting, after 15 minutes venous occlusion and the increment are shown in Table 10.

Comment.

Venous occlusion is associated with a measurable increase in local plasma fibrinolytic activity and therefore forms the basis of a useful test.

Because the venous occlusion test is subjectively unpleasant and the discomfort is to a large extent time-related, a shortened form of the more usual 15-20 minute test was investigated.

5 minute venous occlusion in healthy males does result in an increase in plasma fibrinolytic activity but the mean increment is relatively small and has a wide standard deviation due to the wide range of responses

TABLE 10.

	No. of subjects	RESTING mm lysis		OCCLUDED mm lysis		INCREMENT mm lysis	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
5 MIN. TEST.							
1.	51	11.4	2.5	15.5	5.5	4.1	4.1
2.	51	11.7	2.3	15.7	4.8	4.0	3.4
15 MIN. TEST.							
1.	105	12.2	2.5	23.2	6.5	11.0	5.6
2.	78	12.0	2.3	23.0	6.7	10.9	5.4

The effect of 5 minutes and 15 minutes venous occlusion on plasma fibrinolytic activity in healthy males. The mean fibrinolytic activities resting, after occlusion and the increment (occluded-resting) are shown for each two test points (1 and 2).

observed - the increment varying from zero to 14.5 mm at test point 1 and from zero to 10.2 mm at test point 2. Fifteen minutes venous occlusion results in a much greater increase in fibrinolytic activity with a relatively lower standard deviation.

In spite of the discomfort, therefore it was decided to use the fifteen minute venous occlusion test for all further studies.

REPRODUCIBILITY OF THE 15 MINUTE VENOUS OCCLUSION TEST.

Venous occlusion tests may be subject to a great many variables and it is extremely important that every aspect of the test is meticulously standardised. The standardised 15 minutes venous occlusion test used in this study is described in detail in Chapter V.

Blood Samples and Laboratory Methods.

Blood was collected and plasma prepared as already described in this Chapter.

Full blood counts were carried out automatically using a Coulter S full blood counter (Coulter Electronics Ltd). Fibrinolytic activity in plasma was measured as already described using euglobulin fractions on plasminogen rich fibrin plates (Chapter V).

Statistical Analysis.

Student's paired t test was used to examine the paired samples collected (Chapter V).

Subjects Studied.

The subjects studied were all males in the age range 30-59 years. They belonged basically to two broad groups.

a) healthy male controls.

b) men with angiographic evidence of C.H.D.

For details of the criteria used to select these subjects see Chapters VII (controls) and VIII (C.H.D patients.

Results

The results are shown for the mixed group of all subjects (controls plus C.H.D. patients), controls alone and C.H.D. patients alone. In each case the mean results, resting and occluded, are shown for Test 1 and Test 2. Student's paired t test was carried out on paired results (Test 1 V Test 2) and the r value shown along with their corresponding t and P values.

Full Blood Count Results.

The resting and occluded levels of RBC, haematocrit and WBC are reproducible (the levels found at Test 1 being highly significantly related to the levels found at Test

2) - see Tables 11 (RBC), 12 (haematocrit) and 13 (WBC).

This was true for the mixed population and for the separate groups of controls and C.H.D. patients.

Plasma Fibrinolytic Activity Results.

The resting and occluded levels of plasma fibrinolytic activity are reproducible (the levels found at Test 1 being highly significantly related to the levels recorded at Test 2) - see Table 14.

Comment.

Many variables may influence the venous occlusion test. Great care has been taken to develop a standardised 15 minute venous occlusion test (see Chapter V). When applied with meticulous care it can be demonstrated that this test is highly reproducible.

THE DEVELOPMENT OF PLASMA FIBRINOLYTIC ACTIVITY DURING VENOUS OCCLUSION.

As has been shown already, there is a considerable degree of difference between the increase in fibrinolytic activity produced by 5 minutes venous occlusion and the increase produced by 15 minutes occlusion.

In a small group of healthy individuals, serial samples of blood were collected during venous occlusion after 5 minutes, 10 minutes and 15 minutes and the fibrinolytic activity measured.

TABLE 11.

	TEST 1		TEST 2					
RBCx10^{12}/L	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
ALL SUBJECTS:								
Resting	5.00	.37	4.97	.37	163	.727	13.44	<.0005
Occluded	6.04	.43	6.03	.42	151	.651	10.46	<.0005
CONTROLS:								
Resting	5.01	.37	4.96	.33	72	.715	8.56	<.0005
Occluded	6.06	.41	6.07	.41	73	.719	8.71	<.0005
CHD PATIENTS:								
Resting	4.99	.37	4.97	.39	91	.743	10.47	<.0005
Occluded	6.02	.44	5.99	.44	78	.594	6.43	<.0005

The mean RBC values (RBC x 10^{12}/L) recorded resting and after 15 minutes venous occlusion in a mixed population of controls and men with CHD. Each subject was tested twice and the correlation coefficient r (Test 1 V Test 2) is shown along with its t and P value.

TABLE 12.

HCT %	TEST 1 $\bar{x}$	TEST 1 SD	TEST 2 $\bar{x}$	TEST 2 SD	n	r	t	P
ALL SUBJECTS:								
Resting	45.5	3.2	45.5	3.1	163	.674	11.57	<.0005
Occluded	54.9	3.6	54.9	4.4	151	.495	6.95	<.0005
CONTROLS:								
Resting	45.3	3.4	45.0	2.8	73	.663	7.47	<.0005
Occluded	54.8	4.0	54.2	4.3	72	.583	6.00	<.0005
CHD PATIENTS:								
Resting	45.7	3.0	46.0	3.2	90	.694	9.04	<.0005
Occluded	55.0	3.3	55.4	4.3	79	.405	3.89	<.0005

The mean haematocrit (HCT %) values recorded resting and after 15 minutes venous occlusion in a mixed population of controls and men with CHD. Each subject was tested twice and the correlation coefficient r (Test 1 V Test 2) is shown along with its t and P value.

TABLE 13.

WBCx10^9/L	Test 1 $\bar{x}$	Test 1 SD	Test 2 $\bar{x}$	Test 2 SD	n	r	t	P
ALL SUBJECTS:								
Resting	6.79	1.67	6.71	1.86	162	.761	14.81	<.0005
Occluded	6.91	1.75	6.92	2.23	151	.716	12.50	<.0005
CONTROLS:								
Resting	6.45	1.59	6.15	1.79	72	.680	7.76	<.0005
Occluded	6.64	1.53	6.29	1.78	73	.675	7.71	<.0005
CHD PATIENTS:								
Resting	7.06	1.69	7.16	1.81	90	.805	9.27	<.0005
Occluded	7.17	1.90	7.50	2.46	78	.728	9.27	<.0005

The mean WBC values (WBC x 10^9/L) recorded resting and after 15 minutes venous occlusion in a mixed population of controls and men with CHD. Each subject was tested twice and the correlation coefficient *r* (Test 1 V Test 2) is shown along with its t and P value.

TABLE 14.

	TEST 1		TEST 2					
Lysis-mm	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
ALL SUBJECTS:								
Resting	11.6	2.5	11.6	2.2	174	.501	7.60	<.0005
Occluded	23.2	7.4	23.6	6.8	162	.580	9.02	<.0005
Increment	11.5	6.3	11.8	5.9	161	.529	7.87	<.0005
CONTROLS:								
Resting	12.2	2.4	12.0	2.3	77	.616	6.77	<.0005
Occluded	22.9	6.3	22.9	6.7	78	.619	6.86	<.0005
Increment	10.6	5.3	10.8	5.5	77	.520	5.27	<.0005
CHD PATIENTS:								
Resting	11.2	2.5	11.3	2.0	97	.376	3.95	<.0005
Occluded	23.6	8.3	24.2	6.9	84	.559	6.10	<.0005
Increment	12.3	7.0	12.7	6.1	84	.521	5.53	<.0005

The mean diameter of lysis (mm) recorded resting, after 15 minutes venous occlusion and the increment (occluded-resting) in a mixed population of controls and men with CHD. Each subject was tested twice and the correlation coefficient r (Test 1 V Test 2) is shown along with its t and P value.

Test Procedure.

Each subject was fasted from 9pm on the evening prior to testing. They were admitted to a hospital ward at 8.30am on the morning of testing and rested supine on a bed for 20-30 minutes (see Chapter V).

An indwelling 21 SWG "butterfly" needle was introduced into a vein in each antecubital fossa. Without the use of a tourniquet, resting blood samples were collected from each arm. Thereafter, a sphygmomanometer cuff was applied to each upper arm and inflated to a pressure midway between systolic and diastolic. Stop watches were started and the cuffs were kept carefully inflated to the mid-pressure.

From the left arm, serial samples of venous blood were collected after 5 minutes, 10 minutes and 15 minutes occlusion.

From the right arm a single sample was collected after 15 minutes occlusion.

To keep the indwelling needles patent, after each sample had been collected, normal saline was injected to fill the needle and connecting tube.

Blood Samples and Laboratory Methods.

At each sample point, resting and occluded, andfor each arm, 9 ml venous blood was collected into 1 ml of prechilled 31.3 g/l trisodium citrate. Platelet poor plasma was prepared from the citrated sample as described

TABLE 15.

Subject number	RIGHT ARM		LEFT ARM			
	Diameter of Lysis - mm.					
	Resting	15 min.	Resting	5 min.	10 min	15 min
1	13.3	19.6	13.5	15.7	16.5	18.5
2	14.1	26.4	14.3	15.2	18.4	24.7
3	11.7	19.5	11.6	13.3	14.5	18.3
4	12.4	25.8	12.8	16.1	17.9	24.9
$\bar{x}$	12.9	22.8	13.1	15.1	16.8	21.8

Fibrinolytic activity (diameter of lysis - mm) measured resting and after venous occlusion of each arm in four female volunteers. The left arm of each subject was sampled at 5 minute intervals up to 15 minutes and the right arm once only - at 15 minutes.

in Chapter V.

Ethical permission was sought and granted for this study by the Greater Glasgow Health Boards, Eastern District Ethical Committee.

Plasma fibrinolytic activity was measured as already described using euglobulin fractions on plasminogen rich fibrin plates (Chapter V).

Subjects Studied.

Four healthy female volunteers aged between 25 and 35 years were examined. They had no known medical problems and were taking no drugs. They were all non-smokers and all had normal blood pressure and fasting plasma lipid and glucose levels. They were not pregnant, were not using the contraceptive 'pill' and were tested at a standard point in their menstrual cycles (between days 21-28).

Results.

The fibrinolytic activity detected in each sample of platelet poor plasma prepared from each of the four subjects tested is shown in Table 15.

Comment.

Robertson et al (1972) examined serial blood samples collected at 5 minute intervals during venous occlusion from 18 volunteers and reported that the samples showed a

linear increase in fibrinolytic activity up to 25 minutes. The results from the very small number (4) of volunteers reported here also demonstrate that fibrinolytic activity continues to increase with venous occlusion - at least up to 15 minutes. In these four subjects, the fibrinolytic activity developed after 15 minutes venous occlusion was less in the serially sampled left arm than in the right arm. It could be suggested that the development of fibrinolytic activity with venous occlusion is not related to time alone but also depends on the many factors which determine the effect of a standardised external compression on the venous circulation. As already mentioned in this Chapter the changes in venous blood flow caused by compression probably do not begin to "level off" for at least 5 minutes.

Serial sampling itself, however, may have influenced the development of fibrinolytic activity observed in this experiment. It was spontaneously reported by all of the volunteers, that the discomfort in the serially sampled left arm was very much less than in the right arm. Withdrawal of each of the serial samples produced great relief locally in the left arm. Although the observed external compression pressure remained unaltered by sampling, obviously the actual pressure inside the vein must have changed with sampling.

DISCUSSION.

Spontaneous resting levels of fibrinolytic activator in the plasma are often low and their measurement may yield conflicting results which do not readily permit inter-individual comparisons. The rapid rate of increase in the plasma plasminogen activator levels in response to physiological stimuli suggests that the activator is released from a store. Fibrin autography studies have demonstrated that this store is, at least in part, located in the endothelial cells of the small veins, Isacson (1971) and it is postulated that this store has an important role in the defence against thrombosis. The release of plasminogen activator in response to stimulation allows a more dynamic assessment of the fibrinolytic system than measurement of spontaneous resting activator levels and provides an index of the endothelial fibrinolytic reserve.

Measurement of the local response to venous occlusion of the limbs is a sensitive, simple and clinically applicable method of estimating 'fibrinolytic capacity'.

According to Robertson et al (1972), the fibrinolytic response to venous occlusion increases up to 25 minutes. We were able to demonstrate that fibrinolytic activity continues to increase at least up to 15 minutes venous occlusion. Venous occlusion is however painful and we felt it unreasonable to expect ill patients to accept

repeated prolonged venous occlusion tests.

When the period of time of occlusion is reduced a less than maximal response occurs. Walker et al (1976) have suggested that a 5 minute venous occlusion test of fibrinolytic potential may be clinically useful. Certainly this test may identify non-responders but it is relatively insensitive and probably therefore of limited use in identifying less profound defects.

Interestingly the increases noted in red cell count and haematocrit with venous occlusion are not reflected by similar increases in white cell count or platelet count. Since there is no evidence that venous occlusion for up to 15 minutes causes the veins to become "leaky" it has to be postulated that the apparent relative loss of white cells and platelets is due to other mechanisms. White cells may marginate during venous occlusion or become attracted to the endothelial lining of the vessel. Platelets also may adhere to the endothelium or may form microaggregates.

In spite of the many variables which may influence the venous occlusion test the carefully standardised 15 minute venous occlusion test used in the studies reported in this thesis, has been shown to be highly reproducible.

REFERENCES.

ÅBERG, M., & NILSSON, I.M. (1975). Fibrinolytic response to venous occlusion and vasopressin in health and thrombotic disease, In: Progress in Chemical Fibrinolysis and Thrombolysis, Vol.1. p121, Ed. by Davidson, J.F., Samama, M.M., & Desnoyers, P.C. New York, Raven Press.

AMERY, A., VERMYLEN, J., MAES, H., & VERSTRAETE, M. (1962). Enhancing the fibrinolytic activity in human blood by occlusion of blood vessels. Thrombosis et Diathesis Haemorrhagica. 7, 70.

AMERY, A., VERMYLEN, J., DE VREKER, R.A., VERMYLEN, C., & VERSTRAETE, M. (1965). Enhancing blood fibrinolytic activity by a niacin compound. II. Activation and non-activation. American Journal of Medicine and Science, 249, 66.

CASH, J.D., & ALLEN, A.G.E. (1967). The fibrinolytic response to moderate exercise and intravenous adrenaline in the same subjects. British Journal of Haematology. 13, 376.

CHAMONE, D.A.F., & VERMYLEN, J. (1977). The induction of platelet aggregates in healthy subjects by venous occlusion. Thrombosis and Haemostasis, 38, 132. (Abstract).

CLARKE, R.L., ORANDI, A., & CLIFFTON, E.E. (1960). Induction of fibrinolysis by venous occlusion. Angiology, 11, 367.

CONSTANTINI, R., SPOTTL, F., HOLZNECHT, F., & BRAUNSTEINER, H. (1969). On the role of the stable plasminogen activator of the venous wall in occlusion induced fibrinolytic state. Thrombosis et Diathesis Haemorrhagica. 22, 544.

HOLEMANS, R. (1963). Increase in fibrinolytic activity by venous occlusion. Journal of Applied Physiology. 18, 1123.

HOLZKNECHT, F., SPOTTL, F., & BRAUNSTEINER, H. (1969). Enhancement of fibrinolysis by venous occlusion. Results of long-term thromboelastography and activator determination. Thrombosis et Diathesis Haemorrhagica. 30, 304.

IATRIDIS, S.A., IATRIDIS, P.G., & FERGUSON, J.H. (1966). The role of HF (factor XII) in the pathogenesis of the thrombolytic state induced by venous occlusion. Thrombosis et Diathesis Haemorrhagica. 16, 207.

ISACSON, S. (1971). Low fibrinolytic activity of blood and vein walls in venous thrombosis. Scandinavian Journal of Haematology. (Suppl. 16). 21.

ISACSON, S., & NILSSON, I.M. (1972). Defective fibrinolysis in blood and vein walls in recurrent "idiopathic" venous thrombosis. Acta Chirurgica Scandinavica. 138, 313.

NILSSON, I.M., & ROBERTSON, B. (1968). Effect of venous occlusion on coagulation and fibrinolytic components in normal subjects. Thrombosis et Diathesis Haemorrhagica. 20, 397.

ROBERTSON, B. (1971). On thrombosis, thrombolysis and fibrinolysis. Acta Chirurgica Scandinavica. 421, 42.

ROBERTSON, B.R., PANDOLFI, M., & NILSSON, I.M. (1972). "Fibrinolytic Capacity" in healthy volunteers as estimated from effect of venous occlusion of arms. Acta Chirurgica Scandinavica. 138, 429.

VAN LEEUWEN, A.M. (1964). Base binding power of the plasma proteins. A study of ion protein interaction in human plasma by means of in vivo ultrafiltration and equilibrium dialysis. (Thesis), p90, Amsterdam, Scheltema and Holkema.

WALKER, I.D., DAVIDSON, J.F., & HUTTON, I. (1976). "Fibrinolytic Potential". The response to a 5 minute venous occlusion test. Thrombosis Research, 8, 629.

CHAPTER VII.

FIBRINOLYTIC ACTIVITY AND C.H.D. "RISK FACTORS" -AN EXAMINATION OF POSSIBLE RELATIONSHIPS IN HEALTHY CONTROLS.

INTRODUCTION.

In any study of relationships between fibrinolytic activity (F.A.) and disease, it is critically important to examine the possibility of common associations existing between fibrinolytic activity, the disease process and some other factor or factors. In the specific instance of F.A. and coronary heart disease (C.H.D.) it is important that associations between fibrinolytic activity and C.H.D. "risk factors" be sought.

The term C.H.D. "risk factor" is widely used to describe characteristics found in groups of healthy individuals which are related to a subsequent increased risk of C.H.D. Studies have shown that increasing age and male sex are the two most important non-modifiable "risk factors". As far as modifiable "risk factors" are concerned, the three most important would appear to be hypertension, blood lipids and cigarette smoking - for review see Chapter II.

Various workers have already suggested that reduced

levels of fibrinolytic activity may be associated with some of these C.H.D. "risk factors" (Sweet et al 1966, Meade and North 1977, Meade et al 1977a, 1977b, 1979,) and it is the purpose of this chapter to examine in some detail, the possibility that relationships exist between various aspects of fibrinolysis and C.H.D. "risk factors".

MATERIALS AND METHODS.

Clinical Material.

For the purposes of examining the possibility that relationships may exist between the level of plasma fibrinolytic activity and various coronary heart disease "risk factors", a group of 105 males who had no clinical evidence of coronary heart disease was examined. For the purpose of this thesis these men were designated controls. These controls were drawn from two categories.

Male Volunteers:

Fifty-three healthy male volunteers aged between 30 and 59 years were recruited from the hospital laboratory staff and from the staff of a nearby factory. The nature and the purpose of the study were fully explained to groups of potential volunteers. Volunteers had to be, to the best of their knowledge, healthy and to be on no medication within two weeks preceding examination. Particular care was taken to exclude subjects with diabetes mellitus, known or suspected malignancy, a past history of thrombosis or any history suggestive of

vascular disease either peripheral or coronary arterial.

Because it was felt that none of the available non-invasive techniques of cardiac assessment would irrefutably demonstrate either normality of the coronary arteries or, conversely, the presence of C.H.D., it was considered unethical to subject these men, who had no reason to doubt the health of their coronary vessels, to any form of medical examination (e.g., electrocardiogram) which may have implied to them that specialist cardiological tests were carried out.

The evidence of coronary artery health in these men was therefore based on a careful and detailed medical history and on clinical examination. Predictably, the age distribution in these volunteers was skewed by the much greater number of young men who believed themselves healthy than of men in their forties and fifties.

Normal Coronary Angiogram Subjects:

Even with the most careful selection criteria, there is always a small percentage of patients subjected to coronary artery angiography who are found to have no evidence of occlusive coronary arterial disease. Over the 3 year period for which this study ran, 52 men aged between 30 and 59 years, who were found to have angiographic evidence of normal coronary arteries, were considered suitable for inclusion in the study and willingly gave their informed consent to participate.

(For details of angiography procedure see Chapter VIII).

Patients with diabetes mellitus, peripheral vascular disease or a history of neoplasm were excluded.

Smokers and Non-Smokers.

In some instances, for the purposes of analysis, the group has been subdivided into smokers and non-smokers. The term smoker refers only to the individual's cigarette habit at the time of study - an individual being classified as a smoker if he regularly smoked cigarettes - no matter how few - at the time of study. Non-smokers included life-long non-smokers and ex-smokers who had not smoked for at least six months prior to examination. Subjects who were not currently smoking but who had smoked cigarettes within the preceding 6 months were excluded. Similarly pipe-smokers were excluded from the study.

Normal Lipid; Abnormal Lipid.

Again, in some instances for the purposes of analysis, the group was subdivided into those who where deemed to have a normal plasma lipid profile and those who were deemed to have an abnormal plasma lipid profile. This classification was made on the basis of the normal age related plasma triglyceride and cholesterol levels published by Frederickson et al in 1967 (Table 16). None of the male volunteers had any lipid abnormality which had required investigation. The only lipid abnormality found in this group was (in five individuals) elevated levels of very low density lipoprotein (VLDL). In the group of controls with negative angiograms there was a greater scatter of plasma lipid levels - elevated levels of

TABLE 16.

AGE years	TRIGLYCERIDE mmol/L	TOTAL CHOLESTEROL mmol/L
30 - 39	1.7	3.9 - 7.1
40 - 49	1.8	4.1 - 7.4
50 - 59	2.15	4.4 - 8.0

Lipoprotein limits. Table modified from Fredrickson et al (1967).

TABLE 17.

	30-39 yrs.	40-49 yrs.	50-59 yrs.
VOLUNTEERS:			
Normal lipid			
- non-smokers:	23	7	6
- smokers:	6	1	5
Abnormal lipid			
- non-smokers:	1	1	0
- smokers:	1	1	1
NORMAL ANGIOGRAM:			
Normal lipid			
- non-smokers:	2	6	6
- smokers:	3	15	3
Abnormal lipid			
- non-smokers:	2	2	1
- smokers:	1	9	2

Composition of control group showing the numbers of men in each subgroup.

triglyceride and VLDL being more common in this group (17 men) than in the volunteers. Two also had elevated cholesterol levels for their age. None of these men however were thought to have severe abnormality requiring further investigation or drug treatment. The classification "abnormal lipids" therefore refers to a subgroup of men found to have elevated levels of plasma lipid for their age or abnormality on electrophoresis, but does not include any individual with severe abnormality requiring investigation or drug treatment.

Table 17 shows the composition of the control group with the numbers recruited in each subgroup decade by decade.

Laboratory Methods.

Fibrinolytic Activity.

Using a fibrin plate method, plasma fibrinolytic activity was measured in euglobulin fractions prepared from plasma collected from resting subjects and after fifteen minutes venous occlusion. For details of sample collection and preparation, the venous occlusion test, preparation of the euglobulin fraction, the fibrin plate assay method and statistical methods see Chapter V.

Plasma Lipid Levels.

On two separate occasions at least one month apart, fasting blood lipid levels were measured. Prior to testing, the subjects were fasted for twelve hours. Each test was carrid out at 9 am with the subjects resting supine for at least 30 minutes prior to taking the venous

blood samples. On each occasion, 10 ml of venous blood were mixed with dried sequestrene powder (ethylenediamine tetraacetic acid) in a standard tube.

These samples were immediately dispatched to the Biochemistry Department, Glasgow Royal Infirmary, where plasma was separated to measure total plasma cholesterol and plasma triglycerides using the methods in routine use.

Total plasma cholesterol was measured using the method described by Annan and Isherwood (1967) and triglycerides were measured using a fluorometric assay (Kessler and Lederer 1965, Carlson 1973). Paper electrophoretic determinations were also carried out on each sample.

Ethical Permission.

Ethical permission was sought and granted, for this study, by the Greater Glasgow Health Boards Eastern District Ethical Committee.

Tables.

To facilitate reading, the tables of results in this chapter have been collected together in groups and appear at the end of each relevant section of text.

AGE AND FIBRINOLYTIC ACTIVITY.

The relationship between age and fibrinolytic activity was examined in one hundred and five men aged between 30 and 59 years who had no clinical evidence of coronary heart disease.

These healthy controls were divided into groups decade by decade. (30-39 years, 40-49 years and 50-59 years), according to their age at the last birthday preceding first entry to the study.

Seventy nine of these controls were re-examined one to six months after their initial test. The distribution of controls in each decade subgroup is shown in Table 17.

Results.

The relationship between age and fibrinolytic activity in men with no clinical evidence of coronary heart disease and in the age range of 30-59 years is shown in Table 18.

The 30-39 year olds were found to have significantly greater mean resting fibrinolytic activity than those in the decade 40-49 years. The 50-59 year olds also had greater mean resting fibrinolytic activity than their 40-49 year old counterparts, but this was statistically significant only at Test 2.

After 15 minutes venous occlusion, mean plasma fibrinolytic activity was greater in the 30-39 year olds

and in the 50-59 year olds than in the 40-49 year olds but, with the exception of the difference between the 40-49 year olds and the 50-59 year olds at Test 1, these differences were not statistically significant. No significant differences were noted in the mean fibrinolytic increment between the successive decade groups.

The control group was subdivided into smokers and non-smokers and into those with a normal plasma lipid profile and those with an abnormal plasma lipid profile.

The results of the fibrinolytic tests carried out on the non-smoking, normal lipid controls are shown in Table 19. As in the whole control group, the non-smoking normal lipid 30-39 year old controls had significantly greater mean resting fibrinolytic activity than the correspondng 40-49 year olds. No other significant differences were found in mean plasma fibrinolytic activity between successive decade subgroups in the non-smoking normal lipid controls, resting, post-occlusion or in the increment.

In those controls who had a normal plasma lipid profile and were cigarette smokers, (Table 20) the mean resting fibrinolytic activity was greater in the 50-59 year olds than in 40-49 year olds but this was statistically significant only in those controls tested at Test 2. Although amongst those controls who had normal lipids and who were cigarette smokers, the general trends suggested an increasing response to venous occlusion with increasing age, this was not statistically significant

(Table 20).

The numbers of controls smoking or non-smoking with abnormal lipid profiles were too small to allow examination decade by decade.

Comment.

Increasing age is probably the most powerful predictor of C.H.D. A number of changes in the fibrinolytic system have been reported in relation to age.

In general, plasminogen activator levels have been reported as being unaffected by age, (Sawyer et al 1960, Fearnley et al 1963, Hamilton et al 1974), however some workers have described increasing clot lysis activity with age (Hume 1961, Swan 1963) and have suggested that this may reflect increasing plasminogen activator levels. Meade et al (1977b) however, reported that in men, fibrinolytic activity decreases significantly with age - most of the fall occurring at a steady rate before the age of 45 years.

In the group of healthy men examined in the study reported here, mean resting fibrinolytic activity was shown to be greater in young men in their thirties than in men a decade older. This finding held true for the group as a whole and for the subgroup of non-smokers with normal lipids. The statistical significance of the fall from the level recorded in the thirty year olds to that recorded in the forty year olds was lost in the subgroup of cigarette smokers with normal lipids.

In the whole group, plasma fibrinolytic activity appeared to increase again in the 50-59 year olds compared with the 40-49 year olds. This increase was however not reproducibly significant and may have, at least in part, been due to the fact that lipid abnormality was relatively more common in the 40 year olds than in the 50 year olds (Table 17). No significant difference in fibrinolytic activity between the 40-49 year olds and the 50-59 year olds was observed when the non-smoking, normal lipid controls were studied separately.

No statistically significant relationship between age and fibrinolytic activity post occlusion was found.

Our findings are therefore in broad agreement with the epidemiological data published by Meade et al (1977b) - resting fibrinolytic activity in our group decreased from the thirties to the forties but seems to level off thereafter.

TABLE 18.

		TEST 1.			TEST 2.		
Age-Years		30-39	40-49	50-59	30-39	40-49	50-59
RESTING:	$\bar{x}$	12.7	11.6	12.5	12.4	11.1	12.5
F.A.	n	39	42	24	35	25	18
	SD	2.6	2.4	2.6	2.3	1.5	2.8
	P	<.025	NS		<.01	<.025	
OCCLUDED:	$\bar{x}$	23.0	22.2	25.2	23.0	22.0	24.4
F.A.	n	39	42	24	35	26	18
	SD	5.9	7.2	5.9	6.4	6.8	7.2
	P	NS	<.05		NS	NS	
INCREMENT:	$\bar{x}$	10.3	10.6	12.7	10.6	10.5	11.9
F.A.	n	39	42	24	35	25	18
	SD	5.0	6.4	4.9	4.9	6.2	5.5
	P	NS	<.05		NS	NS	

The relationship between age and fibrinolytic activity (F.A), resting, after 15 minutes venous occlusion and the increment (occluded-resting) in <u>all 'controls'</u>. The mean diameter of lysis (mm) is shown for each decade at Test 1 and at Test 2 along with the P values for comparisons of the means for successive decades.

TABLE 19.

		TEST 1.			TEST 2.		
AGE-YRS:		30-39	40-49	50-59	30-39	40-49	50-59
REST.	$\bar{x}$	13.2	11.4	12.3	13.1	11.1	11.6
F.A.	n	25	13	12	23	9	8
	SD	2.5	2.1	2.5	2.1	1.9	3.3
	P	<.025	NS		<.0125	NS	
OCCL.	$\bar{x}$	24.9	23.2	25.1	24.7	24.0	24.2
F.A.	n	25	13	12	23	10	8
	SD	5.4	6.5	4.9	5.8	4.7	6.1
	P	NS	NS		NS	NS	
INCR.	$\bar{x}$	11.8	11.8	12.8	11.6	12.1	12.5
F.A.	n	25	13	12	23	9	8
	SD	5.0	6.2	4.6	4.5	4.6	3.4
	P	NS	NS		NS	NS	

The relationship between age and fibrinolytic activity (F.A), resting, after 15 minutes venous occlusion and the increment (occluded - resting) in non-smoking, normal lipid controls. The mean diameter of lysis (mm) is shown for each decade at Test 1 and at Test 2 along with the P values for comparisons of the means for successive decades.

TABLE 20.

		TEST 1.			TEST 2.		
AGE-YRS:		30-39	40-49	50-59	30-39	40-49	50-59
REST.	$\bar{x}$	12.5	12.1	12.8	11.8	11.0	14.0
F.A.	n	9	16	8	8	8	6
	SD	3.0	3.2	3.3	2.7	1.0	2.3
	P	NS	NS		NS	<.005	
OCCL.	$\bar{x}$	21.1	23.5	26.1	22.1	22.1	26.6
F.A.	n	9	16	8	8	8	6
	SD	5.7	7.9	7.3	6.8	7.2	7.0
	P	NS	NS		NS	NS	
INCR.	$\bar{x}$	8.6	11.4	13.3	10.3	11.0	12.6
F.A.	n	9	16	8	8	8	6
	SD	4.5	6.2	5.2	5.4	6.5	5.0
	P	NS	NS		NS	NS	

The relationship between age and fibrinolytic activity (F.A) resting and after 15 minutes venous occlusion and the increment (occluded-resting) in cigarette smoking, normal lipid controls. The mean diameter of lysis (mm) is shown for each decade at Test 1 and at Test 2 along with the P values for comparisons of the means for successive decades.

CIGARETTE SMOKING AND FIBRINOLYTIC ACTIVITY.

Plasma fibrinolytic activity, resting and after fifteen minutes venous occlusion, was measured and the increment calculated in 57 non-smoking males aged 30-59 years with no clinical evidence of coronary heart disease and the results compared with the results of the same tests carried out in 48 healthy males aged 30-59 years who were cigarette smokers. Forty four of the non-smokers and 34 of the smokers were re-tested one to six months after their initial fibrinolytic assessment.

Results.

Although within the whole group of controls the cigarette smokers always had very slightly lower mean levels of fibrinolytic activity than the non-smokers (Table 21); no statistically significant differences were found.

The groups of controls - smokers and non-smokers - were subdivided into those who had a normal plasma lipid profile and those who had evidence of a plasma lipid abnormality.

In those men who had a normal plasma lipid profile and who had no clinical evidence of coronary heart disease, mean plasma fibrinolytic activity at rest and after 15 minutes venous occlusion did not differ

significantly between non-smokers and cigarette smokers (Table 22).

The normal lipid controls were examined decade by decade (Table 23). No significant differences were found in any decade subgroup between smokers and non smokers, when mean resting levels of fibrinolytic activity were compared. However, in the 30-39 year old subgroup, cigarette smokers appeared to respond to venous occlusion with less of an increase in fibrinolytic activity than did the non-smokers (Table 23). This latter observation was statistically significant only at the $P < 0.05$ level and only at Test 1.

The numbers of male controls with abnormal plasma lipid profiles were small (Table 24). In contrast to the findings in the control group as a whole and in the controls with normal plasma lipids, in the subgroup of controls with abnormal plasma lipid profiles, the cigarette smokers tended to have higher mean levels of fibrinolytic activity at rest and after venous occlusion than did the non-smokers. This finding could not be confirmed, as insufficient numbers attended at test point 2.

Comment.

The incidence of C.H.D. is higher in smokers than in non-smokers. Opinions differ on the effect of cigarette smoking on plasma fibrinolytic activity. Pozner and Billimoria (1970) reported that the euglobulin lysis time

was shortened in heavy smokers. Other workers however have found that spontaneous plasma fibrinolytic activity is lower in smokers than in non-smokers (Dalderup et al 1970, Meade et al 1977b). Smoking several cigarettes immediately prior to blood sampling is associated with increased fibrinolytic activity due probably to the effect of nicotine on the release of vascular activator (Janzon and Nilsson 1975. It is important therefore that smokers abstain from cigarette smoking for at least 12 hours prior to blood testing.

In the study reported here, no consistently significant differences were found between the smokers and the non-smokers. Where differences in fibrinolytic activity were observed they were usually not significant or, if significant at one test point, failed to reach statistical significance on repeating the test.

This finding is in keeping with the findings of Janzon and Nilsson (1975) who compared fibrinolytic activity (both spontaneous and after venous occlusion) in groups of randomly selected male smokers and non-smokers and found no differences between the groups.

TABLE 21.

F.A.	NON SMOKERS.			SMOKERS.				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1								
Resting	12.2	57	2.4	12.1	48	2.6	0.18	NS
Occluded	23.9	57	5.9	22.3	48	7.1	1.25	NS
Increment	11.7	57	5.4	10.2	48	5.8	1.365	NS
TEST 2								
Resting	12.1	44	2.4	11.8	34	2.1	0.57	NS
Occluded	23.5	45	5.9	22.2	34	7.6	0.872	NS
Increment	11.2	44	4.6	10.4	34	6.4	0.684	NS

Mean plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) at two test points (1 and 2) in all of the controls, non-smokers compared with smokers showing t and P values for comparison of the means at corresponding test points.

TABLE 22.

F.A.	NON SMOKERS.			SMOKERS.				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1								
Resting	12.2	57	2.4	12.1	48	2.6	0.18	NS
Occluded	23.9	57	5.9	22.3	48	7.1	1.25	NS
Increment	11.7	57	5.4	10.2	48	5.8	1.365	NS
TEST 2								
Resting	12.1	44	2.4	11.8	34	2.1	0.57	NS
Occluded	23.5	45	5.9	22.2	34	7.6	0.872	NS
Increment	11.2	44	4.6	10.4	34	6.4	0.684	NS

Mean plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) at two test points (1 and 2) in the controls with normal lipids non-smokers compared with smokers showing t and P values for comparison of the means at corresponding test points.

TABLE 23.

F.A.	NON SMOKERS.			SMOKERS.				
	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
30-39 yrs.								
Rest (1)	13.2	25	2.5	12.5	9	3.0	0.654	NS
Occ. (1)	24.9	25	5.4	21.1	9	5.7	1.823	<0.05
Rest (2)	13.1	23	2.1	11.8	8	2.7	1.419	NS
Occ. (2)	24.7	23	5.8	22.1	8	6.8	1.069	NS
40-49 yrs.								
Rest (1)	11.4	13	2.1	12.1	16	3.2	0.711	NS
Occ. (1)	23.2	13	6.5	23.5	16	7.9	0.093	NS
Rest (2)	11.1	9	1.9	11.0	8	1.0	0.068	NS
Occ. (2)	24.0	10	4.7	22.1	8	7.2	0.681	NS
50-59 yrs.								
Rest (1)	12.3	12	2.5	12.8	8	3.3	0.420	NS
Occ. (1)	25.1	12	4.9	26.1	8	7.3	0.386	NS
Rest (2)	11.6	8	3.3	14.0	6	2.3	1.490	NS
Occ. (2)	24.2	8	6.1	26.6	6	7.0	0.699	NS

Mean plasma fibrinolytic activity (diameter of lysis-mm) resting (rest) and after 15 minutes venous occlusion (occ) at two test points 1 and 2, in controls with normal plasma lipid profiles, decade by decade, comparing smokers with non-smokers showing t and P values for comparison of the means at corresponding test points.

TABLE 24.

F.A.	NON SMOKERS.			SMOKERS.				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Resting	10.5	7	0.70	11.6	15	1.5	1.929	<0.05
Occluded	19.4	7	6.7	19.8	15	6.2	0.127	NS
Increment	9.0	7	6.7	8.2	15	5.6	0.276	NS

Mean plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment in controls with abnormal plasma lipid profiles, non-smokers compared with smokers showing t and P values for comparison of the means recorded at corresponding test points.

PLASMA LIPID LEVELS AND FIBRINOLYTIC ACTIVITY.

In the group of men selected as being healthy and free from clinical evidence of coronary heart disease, fairly wide ranges of plasma cholesterol and plasma triglyceride levels were detected. This was, of course, partly due to the normal age-related variation in plasma lipid levels but also, within the so-called control group, a proportion of subjects with cholesterol or triglyceride levels which fell outwith the normal range for their age was found.

Plasma fibrinolytic activity, resting and after fifteen minutes venous occlusion, was measured and the increment calculated in 104 men aged 30-59 years with no clinical evidence of coronary heart disease. Samples for plasma cholesterol and triglyceride assays were drawn at the time the samples for resting fibrinolytic activity were being collected. Seventy-six of the controls had a second set of fibrinolytic tests and plasma lipid assays carried out one to six months after initial testing.

Relationships between the levels of plasma cholesterol and fibrinolytic activity and plasma triglyceride and fibrinolytic activity were sought.

Results.

When the control group was examined as a whole, the level of resting plasma fibrinolytic activity did not

appear to be related to plasma cholesterol levels (Table 25). However, the level of fibrinolytic activity detected after fifteen minutes venous occlusion and the increment in fibrinolytic activity were found to be statistically significantly negatively related to plasma cholesterol levels (Table 25) - the greater the level of cholesterol, the poorer the fibrinolytic response to venous occlusion.

In the whole group, resting plasma fibrinolytic activity was found to be inversely related to the level of plasma triglyceride but this finding attained statistical significance only in those controls studied at Test 2 (Table 26). Highly significant inverse relationships were found between plasma triglyceride levels and the level of fibrinolytic activity after fifteen minutes venous occlusion and the fibrinolytic increment resulting from occlusion (Table 26).

The control group was subdivided into a group of non-smokers (57 subjects) and into a group of cigarette smokers (47 subjects).

In the subgroup of non-smoking controls, no significant relationship was found between resting plasma fibrinolytic activity and plasma cholesterol level (Table 27) and the statistically significant relationship between plasma cholesterol level and the level of fibrinolytic activity post-occlusion (which had been found in the whole group) was found only at Test 1 and only at the level of $P < 0.05$.

In the non-smoking controls, both the resting plasma fibrinolytic activity and level of fibrinolytic activity

achieved after fifteen minutes venous occlusion were very significantly inversely related to the level of plasma triglyceride (Table 28).

Perhaps predictably, in the subgroup of controls who smoked cigarettes, there was less evidence suggesting a significant relationship between plasma lipid levels and fibrinolytic activity. No significant relationships were found between plasma cholesterol levels and fibrinolytic activity (Table 29). In the smoking controls, no significant relationship was found between plasma triglyceride level and resting fibrinolytic activity but the fibrinolytic response to venous occlusion was statistically significantly inversely related to triglyceride level (although less strongly so than in the non-smoking controls) - Table 30.

Comment.

Several workers have shown that resting plasma fibrinolytic activity is related to plasma lipid levels (Sweet et al 1966, Meade et al 1977b). Sweet et al (1966) found that in primary carbohydrate induced hypertriglyceridaemia, resting plasma fibrinolytic activity was low and Meade et al (1977b) reported that resting plasma fibrinolytic activity was inversely related to both the plasma cholesterol and the plasma triglyceride levels ($P < 0.01$ and $P < 0.001$ respectively). In the study reported here, although the resting fibrinolytic activity was always negatively related to the cholesterol level,

statistical significance was never achieved - perhaps because the group examined was too small.

Plasma fibrinolytic activity at rest was found to be inversely related to plasma triglyceride level. Interestingly this was most convincingly significant in the non-smokers ($P < 0.005$); significant at Test 2 only in the whole control group and not statistically significant in the cigarette smokers.

Spottl et al (1969) and Almer (1975) have found that hypertriglyceridaemia is associated with a poor fibrinolytic response to venous occlusion. In the study reported here, the fibrinolytic response to venous occlusion has been shown to be significantly negatively associated with the plasma triglyceride level and as with the resting fibrinolytic activity the statistical significance is lowest in the group of cigarette smokers.

The increment in plasma fibrinolytic activity in response to venous occlusion was found to be significantly negatively related to plasma cholesterol levels only when the whole group was studied. As with the observations on the resting levels the lack of significance in the smoking and non-smoking subgroups may be due to inadequate population sample size.

Obviously plasma fibrinolytic activity does have an important relationship with the plasma levels of lipoprotein. It is therefore essential to measure plasma lipid levels in a sample taken at the same time as samples taken for fibrinolytic activity assessment. Alterations in an individuals lipid levels from test to test may be

associated with variability in observed fibrinolytic activity. It is essential that blood samples for fibrinolytic activity measurement are collected from fasting subjects.

TABLE 25.

	CHOLESTEROL mmol/L		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	5.6	1.0	12.2	2.6	104	-.160	1.64	NS
Occ.	5.6	1.0	23.3	6.5	104	-.214	2.21	<.025
Incr.	5.6	1.0	11.1	5.6	104	-.174	1.78	<.05
TEST 2.								
Rest.	5.2	0.9	12.1	2.3	75	-.162	1.40	NS
Occ.	5.2	0.9	23.3	6.6	76	-.145	1.26	NS
Incr.	5.2	0.9	11.1	5.4	75	-.191	1.66	<.05

Mean values for cholesterol (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the whole control group at Test 1 and at Test 2 and showing the correlation coefficient r along with its t and P values for comparison of cholesterol and fibrinolytic activity levels at each test point.

TABLE 26.

	TRIGLYCERIDE mmol/L		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	1.8	1.2	12.2	2.6	104	-.107	1.09	NS
Occ.	1.8	1.2	23.3	6.5	104	-.286	3.02	<.0025
Incr.	1.8	1.2	11.1	5.6	104	-.281	2.96	<.0025
TEST 2.								
Rest.	1.7	1.4	12.1	2.3	75	-.263	2.33	<.0125
Occ.	1.7	1.4	23.3	6.6	76	-.394	3.69	<.0025
Incr.	1.7	1.4	11.1	5.4	75	-.361	3.31	<.0025

Mean values for triglyceride (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded - resting) in the whole control group at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of triglyceride and fibrinolytic activity levels at each test point.

TABLE 27.

	CHOLESTEROL mmol/L		LYSIS DIAM. mm					
	x	SD	x	SD	n	r	t	P
TEST 1.								
Rest.	5.4	0.9	12.2	2.4	57	-.208	1.57	NS
Occ.	5.4	0.9	23.9	5.9	57	-.256	1.96	<.05
Incr.	5.4	0.9	11.7	5.4	57	-.184	1.38	NS
TEST 2.								
Rest.	5.0	0.8	12.3	2.4	42	-.106	0.68	NS
Occ.	5.0	1.0	23.9	5.8	43	-.025	0.16	NS
Incr.	5.0	0.8	11.5	4.6	42	-.130	0.84	NS

Mean values for cholesterol (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of cholesterol and fibrinolytic activity levels at each test point.

TABLE 28.

	TRIGLYCERIDE mmol/L		LYSIS DIAM. mm					
	x	SD	x	SD	n	r	t	P
TEST 1.								
Rest.	1.5	0.8	12.2	2.4	57	-.361	2.87	<.005
Occl.	1.5	0.8	23.9	5.9	57	-.349	2.76	<.005
Incr.	1.5	0.8	11.7	5.4	57	-.216	1.64	NS
TEST 2.								
Rest.	1.3	0.7	12.3	2.4	42	-.414	2.88	<.005
Occl.	1.3	0.7	23.9	5.8	43	-.531	4.01	<.0005
Incr.	1.3	0.7	11.5	4.6	42	-.443	3.13	<.0025

Mean values for triglyceride (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of triglyceride and fibrinolytic activity levels at each point.

TABLE 29.

	CHOLESTEROL mmol/L		LYSIS DIAM. mm					
	x	SD	x	SD	n	r	t	P
TEST 1.								
Rest.	5.8	1.0	12.2	2.7	47	-.115	0.78	NS
Occl.	5.8	1.0	22.5	7.1	47	-.147	1.00	NS
Incr.	5.8	1.0	10.3	5.8	47	-.127	0.86	NS
TEST 2.								
Rest.	5.4	0.9	11.9	2.1	33	-.207	1.17	NS
Occl.	5.4	0.9	22.5	7.5	33	-.243	1.39	NS
Incr.	5.4	0.9	10.6	6.4	33	-.218	1.24	NS

Mean values for cholesterol (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded - resting) in the cigarette smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of cholesterol and fibrinolytic activity levels at each test point.

TABLE 30.

	TRIGLYCERIDE mmol/L		LYSIS DIAM. mm					
	x	SD	x	SD	n	r	t	P
TEST 1.								
Rest.	2.2	1.4	12.2	2.7	47	-.047	0.32	NS
Occl.	2.2	1.4	22.5	7.1	47	-.225	1.54	NS
Incr.	2.2	1.4	10.3	5.8	47	-.299	2.10	<.025
TEST 2.								
Rest.	2.2	1.8	11.9	2.1	33	-.219	1.25	NS
Occl.	2.2	1.8	22.5	7.5	33	-.352	2.09	<.025
Incr.	2.2	1.8	10.6	6.4	33	-.342	2.02	<.05

Mean values for triglyceride (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the cigarette smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of triglyceride and fibrinolytic activity levels at each test point.

BLOOD PRESSURE AND FIBRINOLYTIC ACTIVITY.

Plasma fibrinolytic activity resting and after fifteen minutes venous occlusion was measured and the increment calculated in 104 men with no clinical evidence of coronary heart disease. Seventy seven of these male controls were re-tested one to six months after their initial fibrinolytic assessment.

At each visit, resting supine, blood pressure was carefully checked immediately prior to blood sampling.

Results.

When all of the controls were examined together, no significant relationship was found between resting fibrinolytic activity and systolic blood pressure. At Test 1 there was evidence ($P < .05$) that the post occlusion fibrinolytic activity and the fibrinolytic increment were significantly positively related to systolic blood pressure. This finding was not confirmed in those controls examined at Test 2 (Table 31).

At both Test 1 and Test 2, resting plasma fibrinolytic activity was found to be significantly negatively related to diastolic blood pressure ($P < .05$) - the higher the diastolic pressure the lower the resting plasma fibrinolytic activity. Post occlusion fibrinolytic activity did not seem to be related to diastolic blood

pressure. (Table 32).

When the control group was divided into cigarette smokers and non-smokers no reproducible relationships were found between systolic blood pressure and plasma fibrinolytic activity in either the smokers or the non-smokers (Tables 33, 34) but, as in the control group as a whole, the non-smoking controls demonstrated a significant negative relationship between resting plasma fibrinolytic activity and diastolic blood pressure (Table 35). No significant relationships between plasma fibrinolytic activity and diastolic blood pressure were found in the smoking controls (Table 36).

The control group was subdivided into those with normal plasma lipid profiles and those deemed to have plasma lipid abnormalities.

The results of comparing plasma fibrinolytic activity with systolic blood pressure in these subgroups of controls are shown in Tables 37-40 and of comparing plasma fibrinolytic activity with diastolic blood pressure in the subgroups of controls are shown in Tables 41-44.

No reproducible relationships were found between either systolic or diastolic blood pressure and plasma fibrinolytic activity in any of the four subgroups examined. (Normal lipid, non-smokers; normal lipid, smokers; abnormal lipid, non-smokers; abnormal lipid, smokers).

Comment.

Hypertension is recognised as one of the major precursors of atherosclerotic vascular lesions. Meade et al (1977b) demonstrated, that in men resting plasma fibrinolytic activity is not significantly related to systolic blood pressure but is significantly ($P < 0.01$) negatively related to the diastolic pressure.

In the study reported here, no significant and reproducible relationship was found between systolic blood pressure and fibrinolytic activity. Diastolic blood pressure however was significantly negatively related to resting plasma fibrinolytic activity in the control group as a whole. This significant relationship was observed when the non-smokers were observed separately but was not observed in the cigarette smoking controls nor in the controls with abnormal lipid profiles.

TABLE 31.

	SYSTOLIC BP mm Hg		LYSIS DIAM mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	128.9	13.7	12.2	2.6	104	-.031	0.31	NS
Occl.	128.9	13.7	23.1	6.5	104	.164	1.68	<.05
Incr.	128.9	13.7	10.9	5.6	104	.176	1.80	<.05
TEST 2.								
Rest.	127.7	13.5	12.0	2.3	76	-.117	1.01	NS
Occl.	127.6	13.5	22.9	6.7	77	.085	0.74	NS
Incr.	127.7	13.5	10.9	5.5	76	.165	1.44	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the whole control group at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 32.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	83.2	19.0	12.2	2.6	104	-.169	1.73	<.05
Occl.	83.2	9.0	23.1	6.5	104	.087	0.89	NS
Incr.	83.2	9.0	10.9	5.6	104	.178	1.83	<.05
TEST 2.								
Rest.	83.0	9.4	12.0	2.3	76	-.202	1.77	<.05
Occl.	83.0	9.4	22.9	6.7	77	.018	0.16	NS
Incr.	83.0	9.4	10.9	5.5	76	.113	0.98	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the whole control group at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 33.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	130.2	15.0	12.2	2.4	57	.062	0.47	NS
Occl.	130.2	15.0	23.9	5.9	57	.092	0.69	NS
Incr.	130.2	15.0	11.7	5.4	57	.128	0.96	NS
TEST 2.								
Rest.	128.1	13.8	12.1	2.4	44	-.336	2.31	<.025
Occl.	128.0	13.6	23.5	5.9	45	-.261	1.77	<.05
Incr.	128.1	13.8	11.2	4.6	44	-.141	0.93	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 34.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	127.2	11.9	12.1	2.7	47	.156	1.06	NS
Occl.	127.2	11.9	22.2	7.1	47	.236	1.63	NS
Incr.	127.2	11.9	10.1	5.8	47	.217	1.49	NS
TEST 2.								
Rest.	127.2	13.4	11.8	2.1	32	.234	1.32	NS
Occl.	127.2	13.4	22.1	7.7	32	.467	2.90	<.005
Incr.	127.2	13.4	10.3	6.6	32	.473	2.94	<.005

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the cigarette smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparising of systolic pressure and fibrinolytic activity at each test point.

TABLE 35.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	84.0	9.6	12.2	2.4	57	-.230	1.75	<.05
Occl.	84.0	9.6	23.9	5.9	57	.112	0.84	NS
Incr.	84.0	9.6	11.7	5.4	57	.225	1.71	<.05
TEST 2.								
Rest.	82.7	9.5	12.1	2.4	44	-.386	2.71	<.005
Occl.	82.6	9.4	23.5	5.9	45	-.188	1.26	NS
Incr.	82.6	9.5	11.2	4.6	44	-.031	0.20	NS

Mean values for diastolic blood pressure (mm-mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 36.

	DIASTOLIC BP mm Hg $\bar{x}$	SD	LYSIS DIAM. mm $\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	82.3	8.3	12.1	2.7	47	-.102	0.69	NS
Occl.	82.3	8.3	22.2	7.1	47	.036	0.24	NS
Incr.	82.3	8.3	10.1	5.8	47	.093	0.63	NS
TEST 2.								
Rest.	83.5	9.4	11.8	2.1	32	.102	0.56	NS
Occl.	83.5	9.4	22.1	7.7	32	.257	1.46	NS
Incr.	83.5	9.4	10.3	6.6	32	.268	1.53	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in cigarette smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 37.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	128.6	15.0	12.5	2.5	50	8.02	0.06	NS
Occl.	128.6	15.0	24.5	5.5	50	.266	1.91	<.05
Incr.	128.6	15.0	12.1	5.2	50	.281	2.02	<.025
TEST 2.								
Rest.	126.5	13.3	12.3	2.4	40	-.251	1.60	NS
Occl.	126.4	13.2	24.4	5.4	41	-.096	0.60	NS
Incr.	126.5	13.3	11.9	4.3	40	.039	0.24	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid non-smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 38.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	126.8	12.3	12.4	3.1	32	.173	0.96	NS
Occl.	126.8	12.3	23.3	7.3	32	.266	1.51	NS
Incr.	126.8	12.3	10.9	5.7	32	.246	1.39	NS
TEST 2.								
Rest.	124.9	13.4	12.0	2.4	20	.192	0.83	NS
Occl.	124.9	13.4	23.2	7.2	20	.583	3.04	<.005
Incr.	124.9	13.4	11.2	5.9	20	.628	3.43	<.0025

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 39.

	SYSTOLIC BP mm Hg $\bar{x}$	SD	LYSIS DIAM. mm $\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	141.4	10.7	10.5	0.7	7	.293	0.69	NS
Occl.	141.4	10.7	19.4	6.7	7	-.525	1.37	NS
Incr.	141.4	10.7	9.0	6.7	7	-.551	1.48	NS
TEST 2.								
Rest.	143.7	7.5	10.0	1.0	4	-.939	3.88	<.05
Occl.	143.7	7.5	14.6	2.0	4	-.836	2.15	<.05
Incr.	143.7	7.5	4.6	1.6	4	-.481	0.78	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid, non-smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 40.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	128.3	11.2	11.6	1.5	15	.159	0.58	NS
Occl.	128.3	11.2	19.8	6.2	15	.232	0.86	NS
Incr.	128.3	11.2	8.2	5.6	15	.216	0.80	NS
TEST 2.								
Rest.	131.1	13.1	11.3	1.5	12	.570	2.20	<.05
Occl.	131.1	13.1	20.2	8.5	12	.468	1.67	NS
Incr.	131.1	13.1	8.9	7.6	12	.40	1.42	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal_lipid_ smoking_controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 41.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	83.2	9.8	12.5	6.2	50	-.174	1.22	NS
Occl.	83.2	9.8	24.5	5.5	50	.159	1.11	NS
Incr.	83.2	9.8	12.0	5.2	50	.253	1.81	NS
TEST 2.								
Rest.	81.7	9.3	12.3	2.4	40	-.316	2.05	<.025
Occl.	81.7	9.2	24.4	5.4	41	.037	0.24	NS
Incr.	81.7	9.3	11.9	4.3	40	.139	0.86	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid non-smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 42.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	80.5	8.0	12.4	3.1	32	-.094	0.52	NS
Occl.	80.5	8.0	23.3	7.3	32	.036	0.20	NS
Incr.	80.5	8.0	10.9	5.7	32	.091	0.54	NS
TEST 2.								
Rest.	80.8	9.1	12.0	2.4	20	.101	0.43	NS
Occl.	80.8	9.1	23.2	7.2	20	.359	1.63	NS
Incr.	80.8	9.1	11.2	5.9	20	.395	1.82	<.05

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid, smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 43.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	90.0	4.8	10.5	0.7	7	-.430	1.06	NS
Occl.	90.0	4.8	19.4	6.7	7	.761	2.63	<.025
Incr.	90.0	4.8	9.0	6.7	7	.801	3.00	<.0125
TEST 2.								
Rest.	92.5	6.4	10.0	1.0	4	-.796	1.86	NS
Occl.	92.5	6.4	14.6	2.0	4	.063	0.09	NS
Incr.	92.5	6.4	4.6	1.6	4	.426	0.67	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid non-smoking controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 44.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	86.3	7.8	11.6	1.5	15	.078	0.29	NS
Occl.	86.3	7.8	19.8	6.2	15	.352	1.36	NS
Incr.	86.3	7.8	8.2	5.6	15	.369	1.43	NS
TEST 2.								
Rest.	88.0	8.3	11.3	1.5	12	.444	1.57	NS
Occl.	88.0	8.3	20.2	8.5	12	.374	1.28	NS
Incr.	88.0	8.3	8.9	7.6	12	.332	1.11	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal_lipid,_smoking_controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

BODY WEIGHT AND FIBRINOLYTIC ACTIVITY.

Resting plasma fibrinolytic activity and the plasma fibrinolytic activity after fifteen minutes venous occlusion were measured and the increment calculated in 105 control male subjects aged between 30 and 59 years.

On the day of testing each control was carefully weighed. Relationships between gross body weight and plasma fibrinolytic activity were sought.

Results.

Resting plasma fibrinolytic activity, post occlusion fibrinolytic activity and the fibrinolytic increment were all found to be significantly related to gross body weight in the group of 105 control subjects (Table 45).

When the controls were divided into smokers and non-smokers, plasma fibrinolytic activity both resting and after venous occlusion was found to be significantly negatively related to gross body weight (Tables 46-47). These relationships were more evident in the smokers (Table 47) than the non-smokers (Table 46).

Further subdivision of the control group into those who had a normal plasma lipid profile and those with evidence of lipid abnormality revealed that only resting plasma fibrinolytic activity was significantly negatively related to gross body weight in the non-smoking controls

with normal lipid profiles (Table 48), whereas in the smoking controls with normal plasma lipid profiles, both resting and post occlusion plasma fibrinolytic activity were highly significantly negatively related to gross body weight (Table 49).

No relationships were found between plasma fibrinolytic activity and body weight in the very small subgroups of controls with abnormal plasma lipid profiles (Tables 50 and 51).

Comment.

Obesity in itself is not now considered to be one of the major C.H.D."risk factors." It is usually associated with the presence of other,"risk factors" such as hypertension or elevated plasma lipid levels and is a risk because of these associations.

Many authors have examined fibrinolytic activity in obesity and have found it reduced (Fearnley et al 1963, Shaw and MacNaughton 1963, Ogston and McAndrew 1964, Bennett et al 1966, Grace and Goldrick 1968, Warlow et al 1972). Obese subjects have also been reported as having a poor fibrinolytic response to stimuli such as exercise (Ogston and McAndrew 1964) or venous occlusion (Almer 1975).

In the study reported here no attempt was made to divide the controls into groups of those with a normal weight and those with increased weight. No formal assessment of obesity was included in this study but a

number of the controls were evidently mildly or moderately overweight. Gross body weight has been found to be significantly negatively related to plasma fibrinolytic activity both resting and after venous occlusion. As with the relationships between fibrinolytic activity and C.H.D. "risk factors", already discussed in this Chapter, the relationship between fibrinolytic activity and weight is obscured in subjects with elevated plasma trigyceride levels.

TABLE 45.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	165	24	12.2	2.5	105	-.358	3.99	<.0005
Occl.	165	24	23.2	6.5	105	-.303	3.2	<.0025
Incr.	165	24	11.0	5.6	105	-.188	1.9	<.05

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the all of the controls showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 46.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	167	27.5	12.2	2.4	57	-.336	2.65	<.01
Occl.	167	27.5	23.9	5.9	57	-.249	1.91	<.05
Incr.	167	27.5	11.7	5.4	57	-.119	.891	NS

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking controls showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolyticactivity at each test point.

TABLE 47.

	WEIGHT Lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	161	18.9	12.1	2.7	48	-.432	3.25	<.0025
Occl.	161	18.9	22.3	7.1	48	-.462	3.53	<.0005
Incr.	161	18.9	10.2	5.8	48	-.367	2.67	<.01

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the smoking controls showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 48.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	163	19.7	12.5	2.5	50	-.325	2.38	<.0125
Occl.	163	19.7	24.5	5.5	50	-.122	.853	NS
Incr.	163	19.7	12.1	5.2	50	.026	.179	NS

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid, non-smoking controls showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 49.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	159	19.8	12.4	3.1	33	-.504	3.25	<.0025
Occl.	159	19.8	23.5	7.3	33	-.548	3.65	<.0005
Incr.	159	19.8	11.1	5.7	33	-.426	2.62	<.01

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid, smoking controls showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 50.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	197	52.2	10.5	.7	7	-.291	.68	NS
Occl.	197	52.2	19.4	6.7	7	-.259	.60	NS
Incr.	197	52.2	9.0	6.7	7	-.227	.52	NS

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid non-smoking controls showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 51.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	165	16.8	11.6	1.5	15	-.015	.06	NS
Occl.	165	16.8	19.8	6.2	15	-.127	.46	NS
Incr.	165	16.8	8.2	5.6	15	-.137	.50	NS

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid smoking controls showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

DISCUSSION.

As mentioned in Chapter II, a great deal of effort has been expended in the attempt to identify individuals or groups of individuals at particular risk of C.H.D. Increasing age, male sex, hypertension, increased blood lipid levels and cigarette smoking are generally accepted as being the five most important C.H.D. "risk factors."

In this Chapter an attempt has been made to examine the possibility that observed levels of fibrinolytic activity in plasma may be significantly related to those various factors associated with an increased risk of C.H.D.

There is disagreement about possible differences in plasma fibrinolytic activity in men and in women. Cash (1966) reported that spontaneous fibrinolytic activity was higher in females than in males but this has not been corroborated (Hamilton et al 1974). The response to venous occlusion does not seem to vary with sex under the age of 50 years but thereafter females appear to have a significantly greater response (Robertson et al 1972).

In the study reported here no attempt was made to examine the influence of sex on fibrinolytic activity because it was felt that this would in itself have constituted a major study with many ramifications.

Disagreement exists about the relationship between age and fibrinolytic activity. Our observations suggest

that, in healthy men, fibrinolytic activity at rest falls between the ages of 30-39 years and 40-49 years but thereafter plateaus.

Opinions have varied about the effect of smoking on fibrinolytic activity. Certainly cigarettes smoked immediately prior to testing evoke a "nicotine" response with elevated levels of activity detectable. It is therefore important to ensure that subjects being examined understand the importance of abstaining from smoking prior to testing. We were unable to demonstrate any significant differences in fibrinolytic activity at rest or after venous occlusion between healthy cigarette smokers and healthy non-smokers - an observation which concurs with that of the Malmo Group (Janzon and Nilsson 1975).

The study reported here confirms the findings of Meade et al (1977b) that plasma fibrinolytic activity is negatively related to plasma lipid levels - most strongly to the plasma level of triglyceride. Recently Andersen et al (1982) have also reported that there is a highly significant correlation between fibrinolytic activity and triglyceride levels and furthermore that dietary manipulation resulting in normalisation of triglyceride levels is associated with increased fibrinolytic response to venous occlusion.

Relationships have been reported between plasma fibrinolytic acitivity and blood pressure levels (Meade etal 1977b). We have confirmed that there is a significant negative relationship between fibrinolytic activity and diastolic blood pressure.

Although no formal assessment of obesity was made, a strong negative relationship between gross body weight and fibrinolytic activity was observed.

It seems that, at least in males the level of fibrinolytic activity detectable in plasma and the fibrinolytic response to the stimulus of venous occlusion does vary in relation to a number of the accepted C.H.D. "risk factors". The most striking relationship is between triglyceride level and fibrinolytic activity but the levels of other lipids may be important. The association between triglyceride level and fibrinolytic activity appears so strong that, where the plasma triglyceride level is abnormally high, the relationships between fibrinolytic activity and other CHD "risk factors" may be obscured. Diastolic blood pressure and age also seem to be of some influence. Whilst cigarette smoking in itself was not found to be related to the level of fibrinolytic activity it remains likely that smoking may influence fibrinolytic activity indirectly by its interaction with other "risk factors."

REFERENCES.

ALMER, L.O. (1975) Effect of obesity on endogenous fibrinolytic activity in diabetes mellitus. Journal of Medicine, 6, 351.

ANDERSON, P, ARNESEN, H, & HJERMANN, I. (1982). Increased fibrinolytic capacity after intervention on diet in healthy coronary high risk men. Haemostasis 2, Supplement 1, 39 (abstract).

ANNAN, I.W., & ISHERWOOD, D.M. (1967). An automated method for the direct determination of total serum cholesterol. Journal of Medical Laboratory Technology. 24, 202.

BENNETT, N., OGSTON, C., McANDREW, G., & OGSTON, D. (1966). Studies on the fibrinolytic enzyme system in obesity, Journal of Clinical Pathology, 19, 241.

CARLSON, K. (1973). Lipoprotein fractionation. Journal of Clinical Pathology. 26. Suppl. (ACP) 5, 32.

CASH, J.D. (1966). Effect of moderate exercise on the fibrinolytic system in normal young men and women, British Medical Journal, 2, 502.

DALDERUP, L.M., ZWARTZ, J.A., KELLER, G.H.M., SCHOUTEN, F., & van HAARD, W.B. (1970). Serum lipids, typing, fibrinolysis, and smoking, British Medical Journal, 3, 223.

FEARNLEY, G.R., CHAKRABARTI, R., & AVIS, P.R.D., (1963). Blood fibrinolytic activity in diabetes mellitus and its bearing on ischaemic heart disease and obesity. British Medical Journal, 1, 921.

FREDERICKSON, D.S., LEVY, R.I., & LEES, R.S. (1967). Fat transport in lipoproteins as an integrated approach to mechanisms and disorders. New England Journal of Medicine, 276, 34.

GRACE, C.S., & GOLDRICK, R.B. (1968). Fibrinolysis and body build, Journal of Atherosclerosis Research, 8, 705.

HAMILTON, P.J., DAWSON, A.A., OGSTON, D., & DOUGLAS, A.S. (1974). The effect of age on the fibrinolytic enzyme system, Journal of Clinical Pathology, 27, 326.

HUME, R., (1961). The relationship to age and cerebral vascular accidents of fibrin and fibrinolytic activity. Journal of Clinical Pathology, 14, 167.

JANZON, L., & NILSSON, I.M., (1975). Smoking and fibrinolysis. Circulation, 51, 1120.

KESSLER, S., & LEDERER, H. (1965). In Automation in analytical chemistry. p.341, Ed. by Skeggs, L.T. New York,

MEADE, T.W., & NORTH, W.R.S. (1977). Population-based distributions of haemostatic variables. British Medical Bulletin, 33, 283.

MEADE, T.W., CHAKRABARTI, R., & NORTH, W.R.S. (1977a). Fibrinolytic activity in health and vascular disease. British Medical Journal, 1, 837.

MEADE, T.W., CHAKRABARTI, R., & NORTH, W.R.S. (1977b). Associations between fibrinolytic activity and other variables in an industrial population. In Atherosclerosis, metabolic, morphologic and clinical aspects. Advances in Experimental Medicine and Biology, Vol.82. p.219. Ed. by Manning G.W. & Haust, M.D. New York, Plenum Press.

MEADE, T.W., CHAKRABARTI, R, HAINES, A.P., NORTH, W.R.S, & STIRLING, Y. (1979). Characteristics affecting fibrinolytic activity and plasma fibrinogen concentrations. British Medical Journal, 1, 153.

OGSTON, D., & McANDREW, G.M. (1964). Fibrinolysis in obesity. Lancet, 2, 1205.

POZNER, H., & BILLIMORIA, J.D. (1970). Effect of smoking on blood-clotting and lipid and lipoprotein levels. Lancet, 1, 1318.

ROBERTSON, B., PANDOLFI, M., & NILSSON, I.M. (1972). "Fibrinolytic capacity" in healthy volunteers at different ages as studied by standardized venous occlusion of arms and legs. Acta Medica Scandinavica. 191, 199.

SAWYER, D.W., FLETCHER, A.P., ALKJAERSIG, N., & SHERRY, S. (1960). Studies on the thrombolytic activity of human plasma; Journal of Clinicial Investgation. 39, 426.

SHAW, D.A., & MacNAUGHTON, D. (1963). Relationship between blood fibrinolytic activity and body fatness. Lancet. 1, 352.

SPOTTL, F., HOLZKNECHT, F., & BRAUNSTEINER, H. (1969). Enhancement of the fibrinolytic acitivity by venous occlusion in patients with primary "carbohydrate-induced" hypertriglyceridemia. Acta Haematologica. 51, 154.

SWAN, H.T. (1963). Fibrinolysis related to age in men. British Journal of Haematology. 9, 311.

SWEET, B., RIFKIND, B.M., & McNICOL, G.P. (1966). The relationship between blood lipids and the fibrinolytic enzyme system. Journal of Atherosclerosis Research. 6, 359.

WARLOW, C.P., McNEILL, A., OGSTON, D., & DOUGLAS, A.S. (1972). Platelet adhesiveness, coagulation and fibrinolytic activity in obesity. Journal of Clinical Pathology. 25, 484.

CHAPTER VIII.

FIBRINOLYTIC ACTIVITY AND C.H.D. "RISK FACTORS" - AN EXAMINATION OF POSSIBLE RELATIONSHIPS IN C.H.D. PATIENTS.

INTRODUCTION.

In Chapter VII evidence was sought to confirm or refute the suggestion that relationships may exist between the level of fibrinolytic activity and coronary heart disease "risk factors". The subjects examined in Chapter VII were all believed to be free of obstructive coronary heart disease (C.H.D.) either on the basis of a lack of clinical symptoms or on the basis of a known "negative" angiogram.

In this Chapter, similar evidence of relationships existing between the level of plasma fibrinolytic activity and C.H.D. "risk factors" is sought in a group of patients with known obstructive coronary heart disease. Since previous studies reporting relationships between various C.H.D. "risk factors" and fibrinolytic activity have not always attempted to take into account the state of the subjects coronary artery health, this Chapter will also include comments on the result of examining a mixed population of males some of whom were known to have coronary heart disease (C.H.D) and others who were thought to be healthy.

MATERIALS AND METHODS.

Clinical Material.

For the purposes of examining relationships between C.H.D. "risk factors" and plasma fibrinolytic activity in subjects with clinically significant C.H.D., a group of men with positive evidence of occlusive coronary artery disease was recruited. These men were designated C.H.D. patients.

C.H.D. Patients:

The patient group was made up of men aged between 30 and 69 years, who had angiographic evidence of coronary heart disease. Coronary angiography was carried out using either the Judkins' (Judkins 1967) or Sone's (Sone and Shirley 1962) technique. Left ventriculography was performed and views of the right coronary artery were obtained in the left anterior oblique, the left lateral and the right anterior oblique positions. Views of the left coronary artery were obtained in these positions and in the posterior anterior position.

Patients with evidence of any other systemic disease were carefully excluded. In particular, patients with evidence, or a history suggestive of, diabetes mellitus, peripheral vascular disease or malignancy were not studied. No patient who had had an invasive procedure within the preceding month was studied and patients who had evidence of myocardial infarction within the preceding

four weeks were not examined. Patients taking medication for any complaint other than their C.H.D. were excluded. Patients taking non-steroidal anti-inflammatory agents, antiplatelet agents, lipid lowering agents, nicotinic acid derivatives or anabolic steroids were not studied.

Initially, it had been the intention also to exclude patients taking β adrenergic blocking agents but it rapidly became evident, that so common is the usage of these agents in patients with known or suspected C.H.D, that it would have proven impossible to recruit enough subjects not taking β adrenergic agents for participation in this study.

Fortunately, however, it was possible to examine in a small, separate, double blind study the levels of plasma fibrinolytic activity in a group of post-myocardial infarction male patients taking a standardised dose of Propranolol and compare them with a similar group of men taking a placebo. (Davidson et al 1979). This small study demonstrated that there were no significant differences in plasma fibrinolytic activity, resting or after fifteen minutes venous occlusion when patients taking Propranolol were compared with patients taking a placebo.

Patients considered suitable for inclusion were interviewed and the study outline was explained to them. All patients included in the study willingly gave their informed consent to participate.

Non Smokers, Smokers; Normal Lipid, Abnormal Lipid.

In some instances, for the purposes of analysis the patient group has been subdivided into cigarette smokers

and non-smokers and into those deemed to have a normal plasma lipid profile and those who had evidence of a plasma lipid abnormality. These classifications were made exactly according to the definitions set out in Chapter VII for subgrouping the Controls.

As in the control group, the "abnormal lipid" subgroup contained individuals with minor lipid abnormality only, those individuals with severe abnormality being excluded from the study. None of the patients studied were taking lipid-lowering drugs.

Where controls have been included in the analysis reported in this Chapter the Controls were as previously described (Chapter VII).

Table 52 shows the composition of the whole group of patients and controls detailing the numbers recruited into each subgroup decade by decade.

Methods.

Fibrinolytic Activity.

Using a fibrin plate method, plasma fibrinolytic activity was measured in euglobulin fractions prepared from plasma collected from resting subjects and after fifteen minutes venous occlusion.

For details of sample collection and preparation, the venous occlusion test, preparation of the euglobulin fraction, the fibrin plate assay method and statistical methods see Chapter V.

Plasma Lipid Levels.

On two separate occasions, at least one month apart,

TABLE 52.

Age - yrs.	30-39	40-49	50-59	60-69
PATIENTS:				
Normal lipid-non-smoker	4	24	43	6
-smoker	2	22	20	4
Abnormal lipid-non-smoker	3	16	10	4
-smoker	4	12	8	0
CONTROLS:				
Normal lipid-non-smoker	25	13	12	0
-smoker	9	16	8	0
Abnormal lipid-non-smoker	3	3	1	0
-smoker	2	10	3	0

Composition of patient and control groups showing the number of men in each subgroup.

fasting blood lipid levels were measured. Prior to testing, the subjects were fasted for twelve hours. For details of procedure and methods used for measuring cholesterol and triglycerides see Chapter VII.

Ethical Permission.

Ethical permission was sought and granted, for this study, by the Greater Glasgow Health Board's Eastern District Ethical Committee.

Tables.

To facilitate reading, the Tables of results in this Chapter have been collected together in groups and appear at the end of each relevant section of text.

AGE AND FIBRINOLYTIC ACTIVITY.

The relationship between age and plasma fibrinolytic activity was examined, firstly in a mixed population of 273 men aged between 30 and 59 years, 168 of whom had angiographic evidence of C.H.D. (patients) and 105 of whom were clinically free of evidence of C.H.D. (controls) and secondly, separately, in the group of 168 patients known to have C.H.D. Too few subjects over the age of 59 years were studied to justify inclusion.

The subjects studied were divided into groups decade by decade, (30-39 years, 40-49 years and 50-59 years) according to their age at the last birthday preceding first entry to the study.

One hundred and sixty five of these subjects were re-examined one to six months after their first tests (79 controls and 86 patients). The distribution of subjects in each decade subgroup is shown in Table 52.

Results.

Mixed Population:

The relationship between age and fibrinolytic activity in all subjects aged 30-59 years is shown in Table 53. The mean resting fibrinolytic activity was significantly lower in 40-49 year olds than in 30-39 year olds and lower than in 50-59 year olds - although this

latter result was statistically significant only in those subjects tested at Test 2.

The mean occluded fibrinolytic activity was greater (but not significantly) in 30-39 year olds than in 40-49 year olds.

The mean occluded fibrinolytic activity in the 50-59 year olds was significantly greater than in the 40-49 year olds. This was associated with a significantly greater mean increment in fibrinolytic activity after 15 minutes venous occlusion in the older subjects.

C.H.D. Patients.

When the patient group was studied separately (Table 54), no differences were found in mean resting fibrinolytic activity between the 30-39 year olds and the 40-49 year olds. The 50-59 year olds had higher mean resting fibrinolytic activity than the younger patients but this reached statistical significance only at Test 2. The mean fibrinolytic activity post occlusion and the mean fibrinolytic increment did not differ significantly between the 30-39 year olds and the 40-49 year olds but the 50-59 year olds did show a statistically significantly greater response to venous occlusion (in terms of both mean occluded fibrinolytic activity and mean increment) than the 40-49 year olds. (Table 54).

As with the control group (Chapter VII), the C.H.D. patients were subdivided into smokers and non-smokers and into groups of those who had a normal plasma lipid profile and those with evidence of plasma lipid abnormality. The number of subjects in each of these subgroups was very

small and only limited comparisons were possible.

The mean plasma fibrinolytic activities resting, after 15 minutes venous occlusion and the increment are shown in Table 55 - Table 58 (Table 55 non-smoking, normal lipid patients; Table 56 smoking, normal lipid patients; Table 57 non-smoking, abnormal lipid patients; Table 58 smoking, abnormal lipid patients).

In none of these four subgroups of patients was there any difference noted in mean fibrinolytic activity (resting, occluded or increment) between successive decade groups which was statistically significant at both Test 1 and Test 2.

Comment.

As already mentioned, age is one of the most important C.H.D. "risk factors". Indeed the influence of age on the incidence of C.H.D. is reflected in the composition of the groups of subjects studied (Table 52) - the patients with C.H.D. belonging more frequently in the age range above 40 years than in the younger range studied.

When a mixed population of healthy men and men with C.H.D. was studied, there was reproducible and significant evidence that resting fibrinolytic activity fell from the level measured in the thirty year olds to that found in the forty year olds - concurring with the observations of Meade et al (1977) and with the findings in the healthy control group reported in Chapter VII.

As in the control group reported in Chapter VII, plasma fibrinolytic activity at rest in the 50-59 year olds was similar to that in the 40-49 year olds.

Interestingly the response to venous occlusion was significantly greater in the 50-59 year olds than in the younger subjects.

When the men with C.H.D. were examined separately, the thirty year olds were no longer found to have significantly greater fibrinolytic activity than the forty year olds. This may be because the group of 30 year olds with C.H.D. was small, but more likely reflects the fact that half of these very young C.H.D. patients had elevated levels of plasma triglyceride.

In the C.H.D. patients, resting fibrinolytic activity and the fibrinolytic response to venous occlusion was greatest in the 50-59 year olds. The reason for this is not entirely clear. The proportion of 50 year olds with elevated plasma lipid levels was lower (18 out of 81) than in any other age group (28 out of 74, 40-49 year olds and 7 out of 13, 30-39 year olds) and the significance of the differences in fibrinolytic activity between the 50 year olds and the younger C.H.D. patients was lost when the patients with normal lipid profiles were examined separately.

TABLE 53.

		TEST 1.			TEST 2.		
Age-Yrs.		30-39	40-49	50-59	30-39	40-49	50-59
REST.	$\bar{x}$	12.2	10.8	11.3	12.3	11.0	11.8
F.A.	n	52	116	105	38	58	68
	SD	2.5	2.8	2.5	2.3	1.9	2.2
	P	<.0025		NS	<.0025		<.025
OCCL.	$\bar{x}$	22.4	21.0	23.5	23.0	22.0	25.1
F.A.	n	50	113	102	38	52	65
	SD	6.8	7.7	7.8	6.3	6.9	6.7
	P	NS		<.005	NS		<.01
INCR.	$\bar{x}$	10.2	10.2	12.2	10.7	10.6	13.3
F.A.	n	50	113	102	38	51	65
	SD	6.1	6.8	6.6	4.9	6.2	5.8
	P	NS		<.025	NS		<.01

The relationship between age and fibrinolytic activity (F.A.) resting after 15 minutes venous occlusion and the increment (occluded-resting) in the whole group of patients and controls. The mean diameter of lysis (mm) is shown for each decade at Test 1 and at Test 2 along with the P values for comparisons of means for succesive decades.

TABLE 54.

		TEST 1.			TEST 2.		
Age-Yrs.		30-39	40-49	50-59	30-39	40-49	50-59
REST.	$\bar{x}$	10.3	10.3	10.9	10.4	10.5	11.6
F.A.	n	13	74	81	3	33	50
	SD	2.3	2.9	2.3	2.1	3.3	3.5
	P	NS	NS		NS	<.05	
OCCL.	$\bar{x}$	18.3	20.2	22.9	21.2	21.7	25.4
F.A.	n	11	71	78	3	28	47
	SD	8.6	8.0	8.2	8.6	7.2	6.6
	P	NS	<.025		NS	<.025	
INCR.	$\bar{x}$	7.8	9.8	12.0	10.8	10.6	13.8
F.A.	n	11	71	78	3	28	47
	SD	8.4	7.0	7.0	6.8	6.3	6.0
	P	NS	<.05		NS	<.025	

The relationship between age and fibrinolytic activity (F.A.) resting after 15 minutes venous occlusion and the increment (occluded-resting) in the C.H.D. patients. The mean diameter of lysis (mm) is shown for each decade at Test 1 and at Test 2 along with the P values for comparisons of means for succesive decades.

TABLE 55.

		TEST 1.			TEST 2.		
Age-Yrs.		30-39	40-49	50-59	30-39	40-49	50-59
REST.	$\bar{x}$	9.9	10.3	11.2	10.4	10.6	12.4
F.A.	n	4	24	43	3	11	27
	SD	1.7	3.0	2.2	2.1	1.3	1.6
	P		NS	NS		NS	<.0025
OCCL.	$\bar{x}$	26.8	21.4	23.7	21.2	22.7	26.5
F.A.	n	3	21	41	3	10	26
	SD	3.5	7.9	8.4	8.6	7.0	6.4
	P		NS	NS		NS	NS
INCR.	$\bar{x}$	16.4	10.9	12.6	10.8	11.9	14.1
F.A.	n	3	21	41	3	10	26
	SD	5.1	6.9	7.2	6.8	6.4	5.9
	P		NS	NS		NS	NS

The relationship between age and fibrinolytic activity (F.A.) resting after 15 minutes venous occlusion and the increment (occluded-resting) in normal lipid, non-smoking C.H.D. patients The mean diameter of lysis (mm) is shown for each decade at Test 1 and at Test 2 along with the P values for comparisons of means for succesive decades.

TABLE 56.

		TEST 1.			TEST 2.		
Age-Yrs.		30-39	40-49	50-59	30-39	40-49	50-59
REST.	$\bar{x}$	10.1	10.3	11.0	-	11.6	10.3
F.A.	n	2	22	20	0	7	12
	SD	0.1	3.5	2.9	-	3.2	1.5
	P		NS	NS		-	NS
OCCL.	$\bar{x}$	19.1	22.1	23.8	-	23.0	26.1
F.A.	n	2	22	19	0	6	11
	SD	8.3	8.9	8.2	-	8.6	6.4
	P		NS	NS		-	NS
INCR.	$\bar{x}$	9	11.8	12.7	-	11.0	15.9
F.A.	n	2	22	19	0	6	11
	SD	8.2	7.6	7.1	-	6.9	6.1
	P		NS	NS		-	NS

The relationship between age and fibrinolytic activity (F.A.) resting after 15 minutes venous occlusion and the increment (occluded-resting) in normal lipid, smoking C.H.D. patients The mean diameter of lysis (mm) is shown for each decade at Test 1 and at Test 2 along with the P values for comparisons of means for succesive decades.

TABLE 57.

		TEST 1.			TEST 2.		
Age-Yrs.		30-39	40-49	50-59	30-39	40-49	50-59
REST.	$\bar{x}$	9.1	10.7	9.5	-	11.3	10.4
F.A.	n	3	16	10	0	9	7
	SD	1.8	2.6	2.4	-	2.2	1.8
	P	NS	NS		-	NS	
OCCL.	$\bar{x}$	13	18.9	18.4	-	19.8	21.8
F.A.	n	3	16	10	0	7	6
	SD	11.7	7.9	7.9	-	7.8	6.2
	P	NS	NS		-	NS	
INCR.	$\bar{x}$	3.9	8.2	8.8	-	8.2	11.4
F.A.	n	3	16	10	0	7	6
	SD	9.9	6.9	6.3	-	5.8	4.9
	P	NS	NS		-	NS	

The relationship between age and fibrinolytic activity (F.A.) resting after 15 minutes venous occlusion and the increment (occluded-resting) in abnormal lipid, non-smoking C.H.D. patients The mean diameter of lysis (mm) is shown for each decade at Test 1 and at Test 2 along with the P values for comparisons of means for succesive decades.

TABLE 58.

		TEST 1.			TEST 2.		
Age-Yrs.		30-39	40-49	50-59	30-39	40-49	50-59
REST.	$\bar{x}$	12.1	9.8	10.3	-	10.2	11.4
F.A.	n	4	12	8	0	6	4
	SD	3.7	2.1	0.8	-	1.9	1.9
	P	NS		NS	-	NS	
OCCL.	$\bar{x}$	14.5	16.2	21.9	-	21	21.1
F.A.	n	3	12	8	0	5	4
	SD	1.3	5.8	7.1	-	6.7	7.9
	P	NS		NS	-	NS	
INCR.	$\bar{x}$	2.4	6.5	11.6	-	10.7	9.7
F.A.	n	3	12	8	0	5	4
	SD	4.2	5.2	6.8	-	7.0	6.6
	P	NS		<0.05	-	NS	

The relationship between age and fibrinolytic activity (F.A.) resting after 15 minutes venous occlusion and the increment (occluded-resting) in abnormal lipid, smoking C.H.D. patients The mean diameter of lysis (mm) is shown for each decade at Test 1 and at Test 2 along with the P values for comparisons of means for succesive decades.

CIGARETTE SMOKING AND FIBRINOLYTIC ACTIVITY.

Evidence for an effect of cigarette smoking on fibrinolytic activity was sought by measuring plasma fibrinolytic activity resting and after 15 minutes venous occlusion and calculating the increment in a group of 289 men aged between 30 and 69 years, 169 of whom were non-smokers and 120 of whom smoked cigarettes. One hundred and twelve of the non-smokers had proven C.H.D. and 57 were believed to be healthy. In the group of smokers there were 72 patients known to have C.H.D. and the remaining 48 were "controls" (Table 52).

Results.

Mixed Population

When all of the non-smokers were compared with all of the smokers, fibrinolytic activity, resting and after 15 minutes venous occlusion was found to be very similar in the two groups (Table 59). The mean fibrinolytic activity at rest and after 15 minutes venous occlusion and the fibrinolytic increment were all slightly less in the cigarette smokers than in the non-smoking group, but these differences never approached statistical significance.

C.H.D. Patients.

When the results found in the non-smoking patients with C.H.D. were compared with the results found in the cigarette smoking patients, mean resting fibrinolytic activity was found to be lower in the smokers than in the

non-smokers but this was statistically significant only at Test 2 and only at the $P < 0.05$ level (Table 60). No other significant differences were found in plasma fibrinolytic activity when all the non-smoking patients were compared with all the smoking patients.

The patients were subdivided into those with a normal plasma lipid profile and those with evidence of plasma lipid abnormality. Within these subgroups, the only statistically significant difference detected between the smokers and the non-smokers was in the subgroup of patients with a normal plasma lipid profile, where resting plasma fibrinolytic activity was found to be significantly greater in the non-smokers than in the smokers - but only at Test 2 (Table 61).

In the subgroup of patients with abnormal plasma lipids, no significant differences were detected in the fibrinolytic activity found in smokers and non-smokers (Table 62).

Comment.

Cigarette smoking is accepted as a major "risk factor" for C.H.D. and we were particularly interested in the possibility that cigarette smoking may be associated with altered fibrinolytic activity. However, no reproducible significant difference has been found in the level of fibrinolytic activity, either at rest or in response to venous occlusion, when cigarette smokers were compared with non-smokers.

TABLE 59.

F.A.	NON-SMOKERS.			SMOKERS.				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1								
Rest.	11.3	169	2.6	11.2	120	2.9	0.217	NS
Occl.	22.7	163	7.7	21.7	120	1.3	1.290	NS
Incr.	11.4	163	6.7	10.5	119	6.6	1.189	NS
TEST 2								
Rest.	11.8	109	2.2	11.3	66	2.1	1.293	NS
Occl.	24.0	104	6.6	22.9	62	7.3	0.960	NS
Incr.	12.1	103	5.5	11.5	62	6.5	0.568	NS

Mean plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) at two test points (1 and 2) in the whole group of patients and controls non-smokers compared with smokers showing t and P values for comparison of the means at corresponding test points.

TABLE 60.

F.A.	NON-SMOKERS.			SMOKERS.				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1								
Rest.	10.8	110	2.6	10.6	72	2.8	0.525	NS
Occl.	22.1	106	8.6	21.3	72	8.0	0.659	NS
Incr.	11.3	106	7.3	10.7	71	7.1	0.562	NS
TEST 2								
Rest.	11.5	65	2.0	10.8	32	2.0	1.668	<0.05
Occl.	24.3	59	7.0	23.9	28	7.0	0.332	NS
Incr.	12.7	59	6.1	12.9	28	6.5	0.156	NS

Mean plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) at two test points (1 and 2) in all the C.H.D. patients non-smokers compared with smokers showing t and P values for comparison of the means at corresponding test points.

TABLE 61.

F.A.	NON-SMOKERS.			SMOKERS.				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1								
Rest.	10.9	77	2.6	10.7	48	3.2	0.495	NS
Occl.	23.3	73	8.4	22.7	48	8.3	0.421	NS
Incr.	12.3	73	7.2	11.9	47	7.1	0.259	NS
TEST 2								
Rest.	11.9	46	1.8	10.8	21	2.2	2.03	<0.025
Occl.	25.5	44	6.7	24.8	18	7.0	0.348	NS
Incr.	13.6	44	6.0	14.0	18	6.5	0.227	NS

Mean plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) at two test points (1 and 2) in the normal lipid, C.H.D patients non-smokers compared with smokers showing t and P values for comparison of the means at corresponding test points.

TABLE 62.

F.A.	NON-SMOKERS.			SMOKERS.				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1								
Rest.	10.3	33	2.4	10.3	24	2.0	0.080	NS
Occl.	19.4	33	8.5	18.5	24	6.7	0.459	NS
Incr.	9.1	33	7.2	8.2	24	6.6	0.489	NS
TEST 2								
Rest.	10.7	19	2.1	10.8	11	1.8	0.094	NS
Occl.	20.8	15	6.9	21.8	10	6.9	0.372	NS
Incr.	10.0	15	5.4	10.9	10	6.4	0.409	NS

Mean plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) at two test points (1 and 2) in the abnormal lipid, C.H.D patients non-smokers compared with smokers showing t and P values for comparison of the means at corresponding test points.

PLASMA LIPID LEVELS AND FIBRINOLYTIC ACTIVITY.

In the population examined, fairly wide ranges of plasma cholesterol and plasma triglyceride levels were recorded. This was partly a manifestation of the normal age-related variation in plasma lipid levels but also the result of inclusion, within the population studied, of a number of subjects with frankly elevated levels of plasma cholesterol or triglyceride.

Plasma fibrinolytic activity resting and after fifteen minutes venous occlusion was measured and the increment calculated in a mixed population of men aged between 30 and 69 years some of whom were healthy and some of whom had positive evidence of C.H.D.

Samples for plasma cholesterol and triglyceride assays were drawn at the time the samples for resting fibrinolytic activity were being collected. Ninety four patients with C.H.D. and seventy five control subjects had a second set of fibrinolytic tests and plasma lipid assays, carried out one to six months after initial testing.

Relationships between the levels of plasma cholesterol and fibrinolytic activity and plasma triglyceride and fibrinolytic activity were sought.

Results.

Mixed Population.

When the total population of men was examined, post-occlusion plasma fibrinolytic activity was found to be significantly negatively related to the plasma cholesterol level at both test points (Table 63). Resting plasma fibrinolytic activity and the fibrinolytic increment were also significantly negatively related to plasma cholesterol levels but only in those subjects examined at Test 2 (Table 63).

The relationship between plasma fibrinolytic activity and plasma triglyceride levels was even more evident. Resting fibrinolytic activity, post-occlusion fibrinolytic activity and the fibrinolytic increment were all highly significantly negatively related to the plasma triglyceride levels. (Table 64).

Patient Group.

When the group of patients with proven C.H.D. was examined separately no significant relationship between plasma fibrinolytic and plasma cholesterol levels were found at Test 1 but in the 94 patients examined at Test 2, resting fibrinolytic activity, post-occlusion fibrinolytic activity and the fibrinolytic increment were all significantly negatively related to plasma cholesterol levels (Table 65). As in the mixed population, the patient group demonstrated strong negative relationships between plasma triglyceride levels and resting plasma

fibrinolytic activity, post-occlusion plasma fibrinolytic activity and the fibrinolytic increment (Table 66).

The patient group was subdivided into those who were current cigarette smokers and non-smokers.

In the subgroup of non-smoking C.H.D. patients, resting plasma fibrinolytic activity, post-occlusion plasma fibrinolytic activity and the fibrinolytic increment were all found to be negatively related to plasma cholesterol levels but as in the patient group as a whole this was statistically significant only in those patients examined at Test 2 (Table 67). In the non-smoking C.H.D. patient negative relationships between plasma triglyceride levels and resting plasma fibrinolytic activity, post-occlusion plasma fibrinolytic activity and the fibrinolytic increment were observed as in the patient group as a whole (Table 68).

In the subgroup of C.H.D. patients who were cigarette smokers, no significant relationships were found between plasma cholesterol levels and plasma fibrinolytic activity (Table 69). The plasma triglyceride levels were found to be significantly negatively related to the fibrinolytic increment but only at the $P < .025$ (Test 1) and $P < .05$ (Test 2) level, and to the post-occlusion plasma fibrinolytic activity but only at the $P < .025$ level and only at Test 1 (Table 70).

Comment.

It seems clear that relationships exist between the level of fibrinolytic activity detected in plasma and the co-existing plasma lipid levels.

Plasma fibrinolytic activity at rest and after venous occlusion is clearly related to the plasma triglyceride level when a mixed population of men is studied. Interestingly however the relationship between resting fibrinolytic activity and triglyceride level is no longer evident when a selected group of male smoking C.H.D. patients are studied.

As in the control group, relationships between plasma fibrinolytic activity and cholesterol levels are less evident.

TABLE 63.

	CHOLESTEROL mmol/L		LYSIS DIAM. mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	5.9	1.2	11.2	2.7	281	-.087	1.47	NS
Occl.	5.9	1.2	22.4	7.7	277	-.103	1.71	<.05
Incr.	5.9	1.2	11.1	6.7	276	-.088	1.46	NS
TEST 2								
Rest.	5.7	1.2	11.6	2.2	169	-.254	3.39	<.0005
Occl.	5.7	1.2	23.8	6.8	160	-.199	2.53	<.01
Incr.	5.7	1.2	12.0	5.9	159	-.158	2.01	<.025

Mean values for cholesterol (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the whole group of patients and controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of cholesterol and fibrinolytic activity at each test point.

TABLE 64.

TRIGLYCERIDE LYSIS DIAM.

	mmol/L		mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	2.0	1.4	11.2	2.7	283	-.102	1.72	<.05
Occl.	2.0	1.4	22.4	7.7	277	-.247	4.23	<.0005
Incr.	2.0	1.4	11.1	6.7	276	-.244	4.16	<.0005
TEST 2								
Rest.	2.0	2.0	11.5	2.6	169	-.403	5.69	<.0005
Occl.	2.0	2.0	23.8	6.8	160	-.303	4.00	<.0005
Incr.	2.0	2.0	12.0	5.9	159	-.244	3.16	<.0025

Mean values for triglyceride (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the whole group of patients and controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of triglyceride and fibrinolytic activity at each test point.

TABLE 65.

	CHOLESTEROL mmol/L		LYSIS DIAM. mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	6.1	1.3	10.7	2.7	179	.026	0.34	NS
Occl.	6.2	1.3	21.8	8.4	173	-.039	0.52	NS
Incr.	6.1	1.3	11.1	7.3	172	-.061	0.81	NS
TEST 2								
Rest.	6.1	1.2	11.2	2.0	94	-.221	2.17	<.025
Occl.	6.2	1.2	24.2	7.0	84	-.314	3.00	<.0025
Incr.	6.2	1.2	12.9	6.1	84	-.282	2.66	<.005

Mean values for cholesterol (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the C.H.D. patients and controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of cholesterol and fibrinolytic activity at each test point.

TABLE 66.

TRIGLYCERIDE LYSIS DIAM.

	mmol/L		mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	2.2	1.5	10.7	2.7	179	-.053	0.71	NS
Occl.	2.2	1.5	21.8	8.4	173	-.220	2.95	<.0025
Incr.	2.2	1.5	11.1	7.3	172	-.234	3.14	<.0025
TEST 2								
Rest.	2.3	2.4	11.2	2.0	94	-.300	3.02	<.0025
Occl.	2.2	2.5	24.2	7.0	84	-.297	2.81	<.005
Incr.	2.2	2.5	12.9	6.1	84	-.241	2.25	<.025

Mean values for triglyceride (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of triglyceride and fibrinolytic activity at each test point.

TABLE 67.

	CHOLESTEROL mmol/L		LYSIS DIAM. mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	6.1	1.2	10.8	2.6	110	-.014	0.15	NS
Occl.	6.1	1.2	22.1	8.6	104	-.055	0.56	NS
Incr.	6.1	1.2	11.3	7.4	104	-.063	0.49	NS
TEST 2								
Rest.	6.1	1.1	11.5	2.0	63	-.265	2.15	<.025
Occl.	6.1	1.1	24.3	7.1	57	-.329	2.58	<.01
Incr.	6.1	1.1	12.7	6.0	57	-.286	2.21	<.025

Mean values for cholesterol (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of cholesterol and fibrinolytic activity at each test point.

TABLE 68.

	TRIGLYCERIDE mmol/L		LYSIS DIAM mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	2.1	1.5	10.8	2.6	110	-.050	0.52	NS
Occl.	2.2	1.6	22.1	8.6	104	-.197	2.03	<.025
Incr.	2.2	1.6	11.3	7.4	104	-.212	2.19	<.025
TEST 2								
Rest.	2.2	2.7	11.5	2.0	63	-.407	3.48	<.0005
Occl.	2.2	2.9	24.3	7.1	57	-.303	2.36	<.0125
Incr.	2.2	2.8	12.7	6.0	57	-.219	1.67	<.05

Mean values for triglyceride (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of triglyceride and fibrinolytic activity at each test point.

TABLE 69.

	CHOLESTEROL mmol/L		LYSIS DIAM. mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	6.1	1.5	10.6	2.8	69	.073	0.60	NS
Occl.	6.2	1.5	21.4	8.0	69	.018	0.15	NS
Incr.	6.2	1.5	10.8	7.2	68	-.060	0.49	NS
TEST 2								
Rest.	6.3	1.5	10.7	1.9	31	-.141	0.77	NS
Occl.	6.3	1.5	23.9	7.1	27	-.304	1.59	NS
Incr.	6.3	1.5	13.2	6.4	27	-.295	1.54	NS

Mean values for cholesterol (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of cholesterol and fibrinolytic activity at each test point.

TABLE 70.

	TRIGLYCERIDE mmol/L		LYSIS DIAM. mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	2.2	1.5	10.6	2.8	69	-.056	0.46	NS
Occl.	2.2	1.5	21.4	8.0	69	-.259	2.19	<.025
Incr.	2.2	1.5	10.8	7.2	68	-.270	2.28	<.025
TEST 2.								
Rest.	2.3	1.7	10.6	1.9	31	.042	0.25	NS.
Occl.	2.4	1.8	21.4	7.1	27	-.299	1.56	NS
Incr.	2.4	1.8	13.2	6.4	27	-.340	1.84	<.05

Mean values for triglyceride (mmol/L) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of triglyceride and fibrinolytic activity at each test point.

BLOOD PRESSURE AND FIBRINOLYTIC ACTIVITY.

Although a range of blood pressures was recorded in the subjects included in this study, no hypotensive individuals were found and, for practical reasons, patients with severe hypertension were excluded. It was considered that a sphgmomanometer cuff inflated to a pressure exceeding 110 mm of mercury would prove intolerably painful if the pressure was maintained for the 15 minutes required for the venous occlusion test. Patients with diastolic blood pressures of more than 100 mm of mercury were therefore excluded.

Plasma fibrinolytic activity resting and after fifteen minutes venous occlusion was measured and the increment calculated in 287 men aged between 30 and 69 years, 182 of whom had angiographic evidence of C.H.D. and 105 of whom were clinically healthy controls. One hundred and seventy one of these men (95 C.H.D. patients and 76 controls) were retested one to six months after this initial fibrinolytic assessment.

At each visit, the subject's resting, supine blood pressure was carefully checked immediately prior to blood sampling.

Results.

Mixed Population.

No significant relationships were found between plasma fibrinolytic activity, resting or after fifteen minutes venous occlusion, and systolic blood pressure but, perhaps not surprisingly, the fibrinolytic increment was found to be significantly positively related to systolic blood pressure ($P < .025$ at Test 1 and $P < .05$ at Test 2 - Table 71).

Resting plasma fibrinolytic activity was found to be highly significantly ($P < .005$) negatively related to the diastolic blood pressure at both test points but no significant relationships were found between either the post occlusion plasma fibrinolytic activity or the fibrinolytic increment and diastolic blood pressure (Table 72).

C.H.D. Patients.

When the patients with angiographic evidence of C.H.D. were examined separately, no significant relationships were found between plasma fibrinolytic activity and systolic blood pressure (Table 73) and although at both test points resting plasma fibrinolytic activity was negatively related to diastolic blood pressure this was statiscally significant only at Test 2 and only at the $P < .05$ level. (Table 74). Diastolic blood pressure was not significantly related to the plasma fibrinolytic activity detected after 15 minutes venous

occlusion.

When the patient group was divided into those who were cigarette smokers and those who were non-smokers, no significant relationships were found between plasma fibrinolytic activity and blood pressure - systolic or diastolic - in either the non-smokers or the smokers (Tables 75-78).

The C.H.D. patient group was further subdivided into those with normal plasma lipid profiles and those with evidence of plasma lipid abnormality. Systolic blood pressure was found to be signficantly positively related to the fibrinolytic increment in those patients who had normal plasma lipids, both in the non-smokers and in the smokers but in each subgroup this was observed at one test point only (Tales 79 and 80). No significant relationships were found between plasma fibrinolytic activity and systolic blood pressure in the patients with abnormal plasma lipid profiles (non-smokers Table 81 or smokers Table 82).

Resting plasma fibrinolytic activity was found to be negatively related to the diastolic blood pressure in those patients with normal plasma lipid profiles (non-smokers Table 83, smokers Table 84) but this attained statistical significance only in the subgroup of patients who smoked and had normal lipids and in them only at Test 1 and at a P value of $<.05$ (Table 84).

No significant relationships were found between plasma fibrinolytic activity and diastolic blood pressure in those patients with abnormal plasma lipid profiles who

were non-smokers (Table 85) or who were smokers (Table 86).

Comment.

As in the control group reported in Chapter VII, no significant and reproducible relationship was found between systolic blood pressure and fibrinolytic activity when a group of men with C.H.D. was examined. In a mixed population of men with C.H.D. and healthy men the relationship between diastolic blood pressure and resting fibrinolytic activity noted in Chapter VII was again found. However, this relationship could not be demonstrated reproducibly significantly when the C.H.D. patients were examined separately.

TABLE 71.

	SYSTOLIC BP. mm Hg		LYSIS DIAM. mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	129.8	14.6	11.2	2.7	287	-.065	1.10	NS
Occl.	130.0	14.6	22.3	7.7	282	.086	1.45	NS
Incr.	130.0	14.6	11.0	6.7	281	.132	2.22	<.025
TEST 2.								
Rest.	129.3	14.4	11.6	2.2	171	-.086	1.13	NS
Occl.	129.9	14.2	23.6	6.8	162	.081	1.03	NS
Incr.	129.9	14.2	11.8	5.9	161	.150	1.91	<.05

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the whole group of patients and controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 72.

	DIASTOLIC BP. mm Hg		LYSIS DIAM. mm.					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1.								
Rest.	85.0	9.6	11.2	2.7	287	-.153	2.61	<.005
Occl.	85.0	9.7	22.3	7.7	282	.019	0.31	NS
Incr.	84.9	9.7	11.0	6.7	281	.081	1.35	NS
TEST 2.								
Rest.	83.9	8.7	11.6	2.2	171	-.203	2.69	<.005
Occl.	83.9	8.8	23.6	6.8	162	-.013	0.17	NS
Incr.	84.0	8.8	11.8	5.9	161	.064	0.81	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis-mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the whole group of patients and controls at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 73.

SYSTOLIC BP LYSIS DIAM.

	mm Hg		mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	130.3	15.0	10.7	2.7	182	-.096	1.31	NS
Occl.	130.7	15.1	21.8	8.3	178	.063	0.83	NS
Incr.	130.6	15.1	11.1	7.2	177	.114	1.52	NS
TEST 2								
Rest.	130.5	15.0	11.3	2.0	95	-.038	0.37	NS
Occl.	131.9	14.5	24.1	6.9	85	.056	0.51	NS
Incr.	131.9	14.5	12.7	6.1	85	.103	0.94	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 74.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	86.0	9.8	10.7	2.7	182	-.096	1.31	NS
Occl.	86.0	10.0	21.8	8.3	178	7.749	0.10	NS
Incr.	85.9	9.9	11.1	7.3	177	.040	0.54	NS
TEST 2								
Rest.	84.6	8.1	11.3	2.0	94	-.183	1.78	<.05
Occl.	84.8	8.1	24.1	6.9	85	-.064	0.59	NS
Incr.	84.8	8.1	12.7	6.1	85	9.84	0.09	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 75.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	130.5	15.8	10.8	2.6	110	-.052	0.55	NS
Occl.	130.8	16.0	22.1	8.6	106	.113	1.16	NS
Incr.	130.8	16.0	11.3	7.3	106	.158	1.63	NS
TEST 2								
Rest.	130.6	15.8	11.5	2.0	65	-.070	0.56	NS
Occl.	132.1	15.6	24.3	7.0	59	.029	0.22	NS
Incr.	132.1	15.6	12.7	6.0	59	.081	0.62	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 76.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	86.1	10.0	10.8	2.6	110	-.039	0.42	NS
Occl.	85.9	10.2	22.1	8.6	106	.066	0.67	NS
Incr.	85.9	10.2	11.3	7.3	106	.087	0.89	NS
TEST 2								
Rest.	84.0	8.1	11.5	2.0	65	-.173	1.39	NS
Occl.	84.0	8.1	24.3	7.0	59	-.076	0.58	NS
Incr.	84.0	8.1	12.7	6.1	59	-.036	0.27	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 77.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	130.1	13.9	10.6	2.8	72	-.171	1.45	NS
Occl.	130.5	13.7	21.3	8.0	72	-.029	0.25	NS
Incr.	130.4	13.8	10.7	7.1	71	.034	0.29	NS
TEST 2								
Rest.	130.2	13.3	10.9	2.1	30	.030	0.16	NS
Occl.	131.5	12.1	23.8	6.8	26	.137	0.68	NS
Incr.	131.5	12.1	12.9	6.4	26	.169	0.84	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 78.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	85.9	9.6	10.6	2.8	72	-.171	1.45	NS
Occl.	86.1	9.7	21.3	8.0	72	-.029	0.25	NS
Incr.	85.9	9.7	10.7	7.1	71	.034	0.29	NS
TEST 2								
Rest.	85.9	8.0	10.9	2.1	30	.030	0.16	NS
Occl.	86.7	8.1	23.8	6.8	26	.137	0.68	NS
Incr.	86.7	8.1	12.9	6.4	26	.169	0.84	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 79.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	128.3	14.3	10.9	2.6	77	.015	0.13	NS
Occl.	128.6	14.6	23.3	8.4	73	.165	1.38	NS
Incr.	128.6	14.6	12.3	7.2	73	.192	1.65	<.05
TEST 2								
Rest.	130.8	15.4	11.9	1.8	46	-.158	1.06	NS
Occl.	131.0	15.7	25.5	6.7	44	-2.38	0.02	NS
Incr.	131.0	15.7	13.6	6.0	44	.055	0.36	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid, non-smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 80.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Resting	130.1	15.0	10.7	3.2	48	-.199	1.37	NS
Occluded	130.7	14.8	22.7	8.3	48	.070	0.48	NS
Increment	130.5	14.9	11.9	7.1	47	9.65	0.07	NS
TEST 2								
Rest.	128.5	13.6	10.8	2.2	20	.131	0.56	NS
Occl.	129.5	11.9	24.4	7.0	17	.443	1.91	<.05
Incr.	129.5	11.9	13.6	6.5	17	.460	2.00	<.05

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid, smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 81.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	135.7	18.0	10.3	2.4	33	-.119	0.67	NS
Occl.	135.7	18.0	19.4	8.5	33	.172	0.97	NS
Incr.	135.7	18.0	9.1	7.2	33	.243	1.40	NS
TEST 2								
Rest.	130.3	17.1	10.7	2.1	19	.083	0.34	NS
Occl.	135.0	15.6	20.8	7.0	15	.270	1.01	NS
Incr.	135.0	15.6	10.0	5.4	15	.326	1.24	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid, non smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 82.

	SYSTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	130.1	11.7	10.3	2.0	24	-.061	0.29	NS
Occl.	130.1	11.7	18.5	6.7	24	.073	0.35	NS
Incr.	130.1	11.7	8.2	6.6	24	.094	0.44	NS
TEST 2								
Rest.	133.8	12.6	10.9	3.6	10	-.251	0.73	NS
Occl.	135.3	12.3	22.6	6.8	9	-.361	1.02	NS
Incr.	135.3	12.3	11.5	6.5	9	-.262	0.72	NS

Mean values for systolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid, smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of systolic pressure and fibrinolytic activity at each test point.

TABLE 83.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	85.5	8.6	10.9	2.6	77	-.057	0.50	NS
Occl.	85.2	8.7	23.3	8.4	73	.025	0.21	NS
Incr.	85.2	8.7	12.3	7.2	73	.042	0.36	NS
TEST 2								
Rest.	82.9	8.3	11.9	1.8	46	-.197	1.33	NS
Occl.	82.6	8.1	25.5	6.7	44	8.025	0.05	NS
Incr.	82.6	8.1	13.6	6.0	44	.043	0.28	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid, non-smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 84.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	85.4	10.2	10.7	3.2	48	-.279	1.97	<.05
Occl.	85.6	10.4	22.7	8.3	48	-.147	1.01	NS
Incr.	85.3	10.3	11.9	7.1	47	-.059	0.40	NS
TEST 2								
Rest.	84.8	8.0	10.8	2.2	20	-.104	0.45	NS
Occl.	85.8	8.3	24.4	7.0	17	.253	1.01	NS
Incr.	85.8	8.3	13.6	6.5	17	.317	1.29	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid, smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 85.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	87.6	12.9	10.3	2.4	33	.019	0.10	NS
Occl.	87.6	12.9	19.4	8.5	33	.196	1.11	NS
Incr.	87.6	12.9	9.1	7.2	33	.225	1.29	NS
TEST 2								
Rest.	86.7	6.9	10.7	2.1	19	.072	0.30	NS
Occl.	88.1	6.6	20.8	7.0	15	.032	0.03	NS
Incr.	88.1	6.6	10.0	5.4	15	.066	0.24	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid, non-smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

TABLE 86.

	DIASTOLIC BP mm Hg		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
TEST 1								
Rest.	86.9	8.3	10.3	2.0	24	.209	1.01	NS
Occl.	86.9	8.3	18.5	6.7	24	.157	0.75	NS
Incr.	86.9	8.3	8.2	6.6	24	.096	0.45	NS
TEST 2								
Rest.	88.4	7.9	11.1	1.9	9	-.390	1.12	NS
Occl.	88.4	7.9	22.6	6.8	9	-.550	1.74	NS
Incr.	88.4	7.9	11.5	6.5	9	-.462	1.38	NS

Mean values for diastolic blood pressure (mm mercury) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid,smoking C.H.D. patients at Test 1 and at Test 2 showing the correlation coefficient r along with its t and P values for comparison of diastolic pressure and fibrinolytic activity at each test point.

BODY WEIGHT AND FIBRINOLYTIC ACTIVITY.

Plasma fibrinolytic activity, resting and after 15 minutes venous occlusion were measured and the increment calculated in 278 men aged between 30 and 59 years, 173 of whom had angiographic evidence of coronary heart disease and 105 of whom were healthy controls.

On the day of testing each subject was carefully weighed. Relationships between gross body weight and plasma fibrinolytic activity were sought.

Results.

Mixed Population:

Both the resting and the post-occlusion plasma fibrinolytic activity were found to be highly significantly negatively related to the gross body weight (Table 87).

C.H.D. Patients.

As in the mixed population, when the C.H.D. patients were studied as a group, both resting plasma fibrinolytic activity and the fibrinolytic activity after venous occlusion were found to be highly significantly negatively related to the gross body weight (Table 88).

When the patient group was divided into smokers and non-smokers, plasma fibrinolytic activity, resting and after fifteen minutes venous occlusion was again found to

be significantly negatively related to gross body weight in both subgroups (Tables 89 and 90).

Further subdivision of the patient group into those with a normal plasma lipid profile and those deemed to have a plasma lipid abnormality, demonstrated that in patients with normal plasma lipid profiles, whether they were cigarette smokers or non-smokers, both resting plasma fibrinolytic activity and the fibrinolytic activity after 15 minutes occlusion were significantly negatively related to gross body weight (Tables 91 and 92)

In patients with abnormal plasma lipid profiles however these associations between body weight and fibrinolytic activity were not evident (Tables 93 and 94).

Comment.

As in the control group reported in Chapter VII, a strong negative relationship between gross body weight and plasma fibrinolytic activity was found in the mixed population and in men with C.H.D. This relationship was lost in patients with lipid abnormality.

TABLE 87.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	161	21.6	11.3	2.7	278	-.257	4.42	<.0005
Occl.	161	21.6	22.6	7.7	272	-.271	4.63	<.0005
Incr.	161	21.7	11.3	6.7	271	-.207	3.47	<.0005

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the whole group of patients and controls showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 88.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	159	19.8	10.8	2.7	173	-.259	3.51	<.0005
Occl.	159	19.8	22.2	8.3	167	-.280	3.75	<.0005
Incr.	159	19.8	11.4	7.2	166	-.223	2.93	<.0025

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the C.H.D. patients showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 89.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	162	19.9	10.9	2.6	106	-.264	2.79	<.005
Occl.	161	19.8	22.6	8.4	100	-.190	1.92	<.05
Incr.	161	19.8	11.7	7.2	100	-.122	1.22	NS

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the non-smoking C.H.D. patients showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 90.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	156	19.5	10.6	2.8	67	-.27	2.29	<.0125
Occl.	156	19.6	21.5	8.2	67	-.45	4.02	<.0005
Incr.	156	19.7	10.8	7.3	66	-.40	3.47	<.0005

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the smoking C.H.D. patients showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 91.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	159	17.6	11.1	2.6	75	-.344	3.13	<.0025
Occl.	158	17.1	24.1	8.0	69	-.25	2.11	<.025
Incr.	158	17.1	12.9	7.0	69	-.149	1.23	<.15

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid non smoking C.H.D. patients showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 92.

	WEIGHT Lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	152	19.9	10.7	3.2	44	-.349	2.41	<.01
Occl.	152	19.9	23.0	8.5	44	-.480	3.54	<.0005
Inc	152	20.1	12.3	7.2	43	-.415	2.92	<.005

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the normal lipid smoking C.H.D. patients showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 93.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	166	24.1	10.3	2.5	31	-.075	0.41	NS
Occl.	166	24.1	19.4	8.5	31	1.09	5.87	<.0005
Incr.	166	24.1	9.1	7.1	31	.027	0.15	NS

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid non smoking C.H.D. patients showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

TABLE 94.

	WEIGHT lbs.		LYSIS DIAM. mm					
	$\bar{x}$	SD	$\bar{x}$	SD	n	r	t	P
Rest.	164	16.7	10.4	2.01	23	.032	.146	NS
Occl.	164	16.7	18.6	6.86	23	-.177	.824	NS
Incr.	164	16.7	8.2	6.73	23	-.189	.886	NS

Mean values for gross body weight (lbs) and plasma fibrinolytic activity (diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in the abnormal lipid smoking C.H.D. patients showing the correlation coefficient r along with its t and P values for comparison of body weight and fibrinolytic activity at each test point.

DISCUSSION.

Examination of the relationship between fibrinolytic activity and C.H.D. "risk factors" in a population including men known to have C.H.D. confirmed that many of the relationships described in the control group reported in Chapter VII could also be demonstrated in a mixed population.

When the C.H.D. patients were examined separately some of the relationships between C.H.D. "risk factors" and fibrinolytic activity were less evident than in the control group.

There was no evident fall in resting fibrinolytic activity with age and the relationship between diastolic blood pressure and resting fibrinolytic activity could be demonstrated at one test point only. The relationship between triglyceride level and fibrinolytic activity however remained evident in the patient group.

As in the control group, no direct relationship was demonstrated between the level of plasma fibrinolytic activity and smoking habit.

REFERENCES.

DAVIDSON, J.F., WALKER, I.D., BEATTIE, J.M., & HUTTON, I. (1979). The effect of long term propranolol therapy on the fibrinolytic response to venous occlusion. In Progress in Chemical Fibrinolysis and Thrombolysis Vol.IV. p109. Ed. by Davidson J.F, Cepelak, V., Samama, M.M. & Desnoyers, P.C. Edinburgh, Churchill Livingstone.

JUDKINS, M.P., (1967). Selective coronary arteriography. Radiology, 89, 815.

MEADE, T.W., CHAKRABARTI, R., & NORTH, W.R.S., (1977). Associations between fibrinolytic activity and other variables in an industrial population. In Atherosclerosis metabolic morphologic and clinical Aspects. Advances in Experimental Medicine and Biology Vol.82. p219. Ed. by Manning, G.W. & Haust, M.D., New York, Plenum Press.

SONE, R.M. JR., & SHIRLEY, E.K. (1962). Cine coronary arteriography. Modern concepts of cardiovascular disease, 31. 735.

CHAPTER IX.

FIBRINOLYTIC ACTIVITY AND CORONARY HEART DISEASE - AN EXAMINATION OF POSSIBLE RELATIONSHIP.

INTRODUCTION.

Many investigators have been interested in the possibility that patients with occlusive arterial disease may have detectable abnormality in their plasma fibrinolytic system (see Chapter IV). In this Chapter an attempt is made to examine the possibility that the existence of coronary heart disease (C.H.D) may have some relationship to the detected level of plasma fibrinolytic activity.

It is of course impossible to identify a group of patients with positive evidence of clinically significant C.H.D. who do not also have detectable C.H.D. "risk factors". In particular in the group reported here, the patients all suffered from at least two major non-modifiable C.H.D."risk factors" - they were adult and they were male.

It has therefore been necessary to compare groups and subgroups of C.H.D. patients and controls in which the distribution of C.H.D. "risk factors" has been matched as carefully as possible. In this way it was hoped to

discover whether or not measurable differences in plasma fibrinolytic activity exist between healthy males and men with C.H.D. - which may be related to the disease itself and not to the coexistence of other factors.

MATERIALS AND METHODS.

Clinical Material.

For the purposes of examining possible relationships between fibrinolytic activity and the existence of C.H.D. two groups of men were recruited.

Healthy Male Controls:

Healthy men aged between 30 and 59 years with no evidence of C.H.D. were recruited as detailed in Chapter VII.

C.H.D. Patients:

Men aged between 30 and 59 years with angiographic evidence of C.H.D. were recruited as detailed in Chapter VIII.

Both groups were subdivided into smokers and non-smokers and into those with normal plasma lipid profiles and those with abnormal plasma lipid levels as detailed in Chapters VII and VIII.

Laboratory Methods.

Fibrinolytic Activity.

Using a fibrin plate method, plasma fibrinolytic activity was measured in euglobulin fractions prepared

from plasma collected from resting subjects and after fifteen minutes venous occlusion. For details of sample collection and preparation, the venous occlusion test, preparation of euglobulin fraction, the fibrin plate assay method and statistical methods see Chapter V.

Plasma Lipid Levels.

For details of the methods used to assess plasma lipid status see Chapter VII.

Full Blood Counts.

On samples collected into standard full blood count tubes containing EDTA (Staynes), the red cells (RBCs) and the white cells (WBCs) were counted and the haematocrit calculated automatically using a Coulter S full blood counter (Coulter Electronics Ltd). Platelets were counted automatically using a Coulter Thrombocounter (Coulter Electronics Ltd).

Ethical Permission.

Ethical permission was sought and granted for this study, by the Greater Glasgow Health Board's Eastern District Ethical Committee.

Tables.

As in Chapters VII and VIII, to facilitate reading, the Tables of results in this Chapter have been collected together in groups and appear at the end of each relevant section of text.

COMPARISON OF PATIENT AND CONTROL GROUPS.

Before examining the results of the plasma fibrinolytic tests carried out on the patient group and comparing them with the fibrinolytic activity of the control group, it may be worthwhile to pause and consider the various similarities and dissimilarities between the two groups.

C.H.D. "Risk Factors".

Sex.

Only males were recruited into both control and patient groups.

Age.

The mean age of the patient group was 49.0 ± 5.8 years and significantly greater therefore than the mean age, 42.5 ± 8.9 years of the control group. This is unfortunate but perhaps an inevitable consequence of the mode of recruitment of each group. Where the numbers permit therefore, comparison between patients and controls include a comparison within each decade subgroup (30-39 years, 40-49 years and 50-59 years).

Smoking Habit.

Cigarette smoking was relatively less common in the patient group (40.4%) than in the control group (45.7%). At first sight this was perhaps a surprising finding but it must be borne in mind that the non-smoking patients included men who had stopped smoking on medical advice.

Although no reproducible difference in the fibrinolytic activity of smokers and non-smokers has been demonstrated, (Chapters VII and VIII) where the numbers permit, separated groups of non-smoking controls and patients and smoking controls and patients have been studied.

Plasma Lipid Levels.

Mean plasma cholesterol levels were highly significantly greater in the patient group than in the controls (Table 95). Mean plasma triglyceride levels were also significantly greater in the patient group than in the controls (Table 95). This is in part a reflection of the fact that more of the patients (32.8%) has plasma lipid levels outwith the normal ranges for their age than did controls (21%) but is also due of course to the greater proportion of older patients examined.

Fibrinolytic activity has been shown to be related to plasma lipid levels, therefore patients and controls with abnormally elevated lipid levels have been separated from the main groups and compared separately.

Blood Pressure.

Mean systolic blood pressures in the patient and control groups were not significantly different (Table 96). The mean diastolic blood pressure was found to be slightly higher in the patients than in the controls (but not reproducibly significantly - Table 96). This slightly higher blood pressure in the patient group reflects the greater proportion of older patients. No severely

hypertensive subjects were examined. No attempt to subgroup patients and controls according to blood pressure was considered necessary.

Body Weight.

The mean gross body weight of the control group (165 ± 24 lbs) was not significantly different from the mean gross body weight of the patients (159 ± 20 lbs). Although fibrinolytic activity was shown to be signficantly related to body weight, because no grossly overweight or underweight subjects were examined, it was considered unnecessary to subgroup patients and controls according to body weight.

The composition of the control and patient groups is shown in Table 97.

Full Blood Count.

Red Cell Count.

The mean red cell count resting and after 15 minutes venous occlusion was the same in the patient and control groups. (Table 98).

Haematocrit.

The mean haematocrit at rest was marginally higher in the patients than in the controls (Table 99). No reproducible difference was found in the mean post occlusion haematocrits.

White Cell Count.

The mean white cell count was significantly higher in the patient group than in the control group both at rest and after 15 minutes venous occlusion (Table 100).

Platelet Count.

Platelet counts were carried out on only a small number of patients (62) and controls (51) and only at rest. The mean platelet count in the patients ($220 \pm 66 \times 10^9/L$) was not signficantly different from the mean platelet count ($205 \pm 51 \times 10^9/L$) in the controls.

Comment.

Both patient and control groups were selected to exclude subjects with evidence of disease other than C.H.D. The age range studied was fairly narrow - no extremes of age being included in either group. The incidence of cigarette smoking was similar in both groups. It is probably not surprising therefore, that there were no obvious differences in red cell count, haematocrit or platelet count when patients and controls were examined. Why there should have been a significant and reproducible difference in the white cell counts of the two groups remains unexplained. Is it merely a reflection of the fact that the C.H.D. patients had chronic disease?

TABLE 95.

	CONTROLS			PATIENTS				
	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Cholest.	5.6	105	.99	6.1	164	1.30	3.697	<.0005
Trigly.	1.8	105	1.20	2.2	164	1.50	2.348	<.01
TEST 2.								
Cholest.	5.2	76	0.92	6.1	83	1.20	5.130	<.0005
Trigly.	1.7	76	1.30	2.1	83	1.40	1.691	<.05

Mean plasma cholesterol (mmol/L) and triglyceride (mmol/L) levels in the control and patient groups at Test 1 and at Test 2 showing the t and P values for comparison of the means at each test point.

TABLE 96.

	CONTROLS			PATIENTS				
	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Systolic	128.8	105	13.7	129.7	168	15.0	0.537	NS
Diastolic	83.2	105	9.0	86.2	168	10.0	2.492	<.01
TEST 2.								
Systolic	127.6	77	13.5	130.5	84	15.0	1.254	NS
Diastolic	83.0	77	9.4	84.7	83	8.3	1.232	NS

Mean systolic and diastolic blood pressures (mm of mercury) in the control and patient groups at Test 1 and at Test 2 showing the t and P values for comparison of the means at each test point.

TABLE 97.

	30-39 years		40-49 years		50-59 years	
	Cont.	Pat.	Cont.	Pat.	Cont.	Pat.
Normal lipid						
Non-smoker	25	4	13	24	12	46
Smoker	9	2	16	22	8	20
Abnormal lipid						
Non-smoker	3	3	3	16	1	10
Smoker	2	4	10	12	3	8

Composition of control (Cont.) and patient (Pat.) groups showing the numbers of men in each subgroup.

TABLE 98.

	CONTROLS			PATIENTS				
RBCx10^{12}/L	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Resting	5.0	96	.39	5.0	180	.40	.971	NS
Occluded	6.1	95	.41	6.0	172	.49	.257	NS
TEST 2.								
Resting	5.0	73	.32	5.0	94	.40	.080	NS
Occluded	6.1	75	.41	6.0	83	.45	.848	NS

Mean red cell counts (x 10^{12}/L) resting and after 15 minutes venous occlusion in the control and patient groups at Test 1 and at Test 2 showing the t and P values for comparison of the means at each test point.

TABLE 99.

	CONTROLS			PATIENTS				
Hct %	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Resting	45.3	96	3.6	45.7	182	3.6	.946	NS
Occluded	55.2	95	4.0	55.0	172	4.3	.435	NS
TEST 2.								
Resting	45.0	74	2.8	45.9	93	3.2	1.988	<.025
Occluded	54.2	74	4.3	55.5	84	4.2	1.967	<.05

Mean haematocrit (%) resting and after 15 minutes venous occlusion in the control and patient groups at Test 1 and at Test 2 showing the t and P values for comparison of the means at each test point.

TABLE 100.

	CONTROLS			PATIENTS				
WBCx10^9/L	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Resting	6.6	96	1.6	7.2	180	1.7	3.215	<.0025
Occluded	6.8	95	1.6	7.3	172	1.9	2.356	<.01
TEST 2.								
Resting	6.1	73	1.8	7.2	93	1.8	3.729	<.0005
Occluded	6.3	75	1.8	7.5	83	2.4	3.682	<.0005

Mean white cell counts (x 10^9/L) resting and after 15 minutes venous occlusion in the control and patient groups at Test 1 and at Test 2 showing the t and P values for comparison of the means at each test point.

FIBRINOLYTIC ACTIVITY.

Results.

All Patients v All Controls.

The mean resting fibrinolytic activity was found to be significantly lower in the whole patient group than in the whole control group at both Test 1 and Test 2 (Table 101). No reproducible difference was found between patients and controls when the fibrinolytic activity was measured post-occlusion (Table 101).

The patient and control groups were divided into decade subgroups (30-39 years, 40-49 years and 50-59 years) and re-examined. In the 30-39 year old both the resting and post occlusion mean fibrinolytic activities were significantly lower in the patients than in the controls at Test 1. Too few patients were examined at Test 2 to allow comment (Table 102).

In the 40-49 year olds and the 50-59 year olds mean resting fibrinolytic activity was found always to be lower in the patients than in the controls (Tables 103 and 104) but in each decade subgroup this was significant only at Test 1. Mean fibrinolytic activity post-occlusion was usually lower in the patients than in the controls but not significantly, and in the 50-59 year olds at Test 1 only (Tables 103 and 104). There were no significant differences in increment.

Normal Lipid Patients v Normal Lipid Controls.

The mean resting fibrinolytic activity was found to be significantly lower in those patients with normal plasma lipid levels than in the controls with normal plasma lipid levels both at Test 1 and at Test 2 (Table 105). No difference was found between the normal lipid patients and controls post-occlusion but the patients achieved greater increments in fibrinolytic activity with occlusion (Table 105).

When the normal plasma lipid patients and controls were divided into decade subgroups (30-39 years, 40-49 years and 50-59 years), mean resting plasma fibrinolytic activity was consistently lower in the patients than in the controls (Tables 106, 107 and 108) although, unfortunately, this reached statistical significance at Test 1 only (too few 30-39 year old patients at Test 2 for comment).

Mean fibrinolytic activity post-occlusion was usually lower in the patients than in the controls (Tables 106, 107 and 108) - except in the 50-59 year olds at Test 2. The post-occlusion differences, and the differences in increment were never statistically significant.

Abnormal Lipid Patients v Abnormal Lipid Controls.

Those patients and controls found to have abnormal plasma lipid profiles were compared.

Mean plasma fibrinolytic activity was lower in the patients than in the controls but this was significant at Test 1 only (Table 109). No significant differences in

the post-occlusion levels or in the increments were observed.

When the abnormal lipid patients and controls were divided into decade subgroups (30-39 years, 40-49 years and 50-59 years), mean fibrinolytic activity at rest was found to be the same in the 30-39 year old patients and controls (one test only - Table 110) and lower in the patients than in the controls in the 40-49 year olds and the 50-59 year olds (Tables 111 and 112). This finding, however, achieved statistical significance only at the P <.01 level and only at Test 1 in the 50-59 year olds.

No significant differences in the post-occlusion levels or in the increments were observed.

Normal Lipid, Non-Smoking Patients v Normal Lipid, Smoking Controls.

The patients and controls found to have normal plasma lipid profiles were divided into cigarette smokers and non-smokers. When all of the non-smoking, normal lipid patients were compared with the non-smoking, normal lipid controls, mean resting plasma fibrinolytic activity was found to be lower in the patients than in the controls but this was statistically significant at Test 1 only (Table 113). No significant differences were found in mean post-occlusion levels or in the mean increments.

The non-smoking normal lipid patients and controls were further examined in decade subgroups (30-39 years, 40-49 years and 50-59 years). With the exception of the 50-59 year olds at Test 2 (Table 116) mean resting fibrinolytic activity was always lower in the non-smoking,

normal lipid patients than in the corresponding controls (Tables 114, 115 and 116). This finding was however significant only in the small group of 30-39 year olds. No significant or reproducible differences were found in the mean post-occlusion levels or the mean increments.

Normal Lipid Smoking Patients v Normal Lipid Smoking Controls.

When all of the smoking, normal lipid patients were compared with the smoking normal lipid controls, mean resting plasma fibrinolytic activity was found to be significantly lower in the patients than in the controls (Table 117). No significant differences were found post-occlusion or in the increments.

The smoking, normal lipid patients and controls were further examined in decade subgroups (30-39 years, 40-49 years and 50-59 years). With the exception of the 40-49 year olds at Test 2, mean resting plasma fibrinolytic activity was always lower in the patients than in the controls - but this reached significance only in the 50-59 year olds at Test 2 (Tables 118, 119 and 120). No significant or reproducible differences were found in the mean post-occlusion levels or mean increments.

The abnormal lipid patients and controls were not examined in subgroups of smokers and non-smokers because the numbers in each subgroup were too small to allow valid comparison (Table 97).

TABLE 101.

F.A.	CONTROLS			PATIENTS				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.2	105	2.5	10.6	167	2.5	5.075	<.0005
Occluded	23.2	105	6.5	21.4	160	8.2	1.904	<.05
Increment	11.0	105	5.6	10.8	160	0.6	0.269	NS
TEST 2.								
Resting	12.0	78	2.3	11.3	86	2.0	2.162	<.025
Occluded	23.0	79	6.7	23.9	78	7.0	0.840	NS
Increment	10.9	78	5.4	12.5	78	6.2	1.770	<.05

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in all patients and in all controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 102.

F.A.	CONTROLS			PATIENTS				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.7	39	2.6	10.3	12	2.3	2.897	<.005
Occluded	23.0	39	5.9	18.3	11	8.5	2.119	<.025
Increment	10.3	39	5.0	7.8	11	8.4	1.224	NS
TEST 2.								
Resting	12.4	35	2.3	10.4	3	2.1	1.447	NS
Occluded	23.0	35	6.4	21.2	3	8.6	0.452	NS
Increment	10.6	35	4.9	10.8	3	6.8	0.089	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in all 30-39 year old patients and in all 30-39 year old controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 103.

F.A.	CONTROLS			PATIENTS				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Resting	11.6	42	2.4	10.3	74	2.9	2.426	<.01
Occluded	22.2	42	7.2	20.2	71	8.0	1.342	NS
Increment	10.6	42	6.4	9.8	71	7.0	0.609	NS
TEST 2.								
Resting	11.1	25	1.5	10.9	33	2.1	0.248	NS
Occluded	22.0	26	6.8	21.7	28	7.2	0.012	NS
Increment	10.5	25	6.2	10.6	28	6.3	0.021	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in all 40-49 year old patients and in all 40-49 year old controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 104.

F.A.	CONTROLS			PATIENTS				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.5	24	2.6	10.9	81	2.3	2.961	<.0025
Occluded	25.2	24	5.9	22.9	78	8.2	1.304	NS
Increment	12.7	24	4.9	12.0	78	7.0	0.448	NS
TEST 2.								
Resting	12.5	18	2.8	11.6	50	1.9	1.535	NS
Occluded	24.4	18	7.2	25.4	47	6.6	0.511	NS
Increment	11.9	18	5.5	13.8	47	6.0	1.149	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in all 50-59 year old patients and in all 50-59 year old controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 105.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.4	83	2.7	10.7	115	2.7	4.36	<.0005
Occluded	24.1	83	6.2	23.0	108	8.2	1.06	NS
Increment	11.7	83	5.4	12.2	108	7.1	0.53	NS
TEST 2.								
Resting	12.3	62	2.4	11.5	60	2.0	1.97	<.05
Occluded	24.0	63	6.0	25.1	56	6.9	0.90	NS
Increment	11.7	62	4.3	13.6	56	6.2	1.88	<.05

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in all normal lipid patients and in all normal lipid controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 106.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	13.0	34	2.6	10.0	6	1.3	2.738	<.005
Occluded	23.9	34	5.6	23.7	5	6.4	0.073	NS
Increment	10.9	34	5.0	13.5	5	6.8	1.003	NS
TEST 2.								
Resting	12.7	31	2.3	10.4	3	2.1	1.706	<.05
Occluded	24.0	31	6.0	21.2	3	8.6	0.746	NS
Increment	11.3	31	4.7	10.8	3	6.8	0.151	NS

Mean fibrinolytic activity (F.A.diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in 30-39 year old, normal lipid patients and in 30-39 year old, normal lipid controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 107.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	11.8	29	2.7	10.3	46	3.2	2.114	<.025
Occluded	23.4	29	7.2	21.8	43	8.3	0.841	NS
Increment	11.6	29	6.1	11.4	43	7.2	0.117	NS
TEST 2.								
Resting	11.1	17	1.5	11.0	18	2.2	0.126	NS
Occluded	23.1	18	5.8	22.8	16	7.3	0.143	NS
Increment	11.6	17	5.4	11.6	16	6.4	0.013	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in 40-49 year old, normal lipid patients and in 40-49 year old, normal lipid controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 108.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.5	20	2.8	11.1	63	2.4	2.076	<.025
Occluded	25.5	20	5.8	23.8	60	8.2	0.872	NS
Increment	13.0	20	4.8	12.6	60	7.1	0.242	NS
TEST 2.								
Resting	12.7	14	3.1	11.8	39	1.9	1.295	NS
Occluded	25.2	14	6.4	26.4	37	6.3	0.591	NS
Increment	12.6	14	4.3	14.6	37	5.9	1.194	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion and the increment (occluded-resting) in 50-59 year old, normal lipid patients and in 50-59 year old, normal lipid controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 109.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	11.3	22	1.4	10.2	51	2.3	1.938	<.05
Occluded	19.7	22	6.2	18.1	51	7.5	0.875	NS
Increment	8.4	22	5.9	7.9	51	6.6	0.343	NS
TEST 2.								
Resting	11.0	16	1.5	10.8	26	1.9	0.292	NS
Occluded	18.8	16	7.7	20.8	22	6.7	0.858	NS
Increment	7.9	16	6.8	9.9	22	5.7	1.013	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in all abnormal lipid patients and all abnormal lipid controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 110.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	10.6	5	0.3	10.6	6	3.1	-	NS
Occluded	16.5	5	2.3	13.7	6	7.5	0.803	NS
Increment	6.0	5	2.5	3.2	6	6.8	0.869	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in 30-39 year old abnormal lipid patients and in 30-39 year old abnormal lipid controls at Test 1 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 111.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	11.0	13	1.2	10.3	28	2.4	1.058	NS
Occluded	19.6	13	6.6	17.8	28	7.1	0.796	NS
Increment	8.6	13	6.6	7.5	28	6.2	0.517	NS
TEST 2.								
Resting	11.0	8	1.7	10.9	15	2.1	0.191	NS
Occluded	19.3	8	8.5	20.3	12	7.1	0.271	NS
Increment	8.3	8	7.6	9.3	12	6.1	0.309	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in 40-49 year old abnormal lipid patients and in 40-49 year old abnormal lipid controls at Test 1 and at Test 2 showing the t and P values for comparison of patients and controls at each test point.

TABLE 112.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.8	4	1.9	9.9	18	1.9	2.764	<.01
Occluded	23.9	4	7.0	20.0	18	7.6	0.959	NS
Increment	11.2	4	5.9	10.1	18	6.5	0.306	NS
TEST 2.								
Resting	11.8	4	1.4	10.8	11	1.8	1.014	NS
Occluded	21.5	4	10.0	21.5	10	6.5	-	NS
Increment	9.8	4	8.9	10.7	10	5.3	0.245	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in 50-59 year old abnormal lipid patients and in 50-59 year old abnormal lipid controls at Test 1 and at Test 2, showing the t and P values for the comparison of patients and controls at each test point.

TABLE 113.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.5	50	2.5	10.9	71	2.5	3.48	<.0025
Occluded	24.5	50	5.5	23.1	65	8.2	1.05	NS
Increment	12.0	50	5.2	12.2	65	7.1	0.11	NS
TEST 2.								
Resting	12.3	40	2.4	11.8	41	1.8	1.16	NS
Occluded	24.4	41	5.4	25.1	39	6.8	0.51	NS
Increment	11.9	40	4.3	13.3	39	6.0	1.18	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in all normal lipid, non-smoking patients and in all normal lipid, non-smoking controls at Test 1 and at Test 2, showing the t and P values for the comparison of patients and controls at each test point.

TABLE 114.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	13.2	25	2.5	9.9	4	1.7	2.477	<.01
Occluded	24.9	25	5.4	26.8	3	3.5	0.577	NS
Increment	11.8	25	5.0	16.4	3	2.9	1.519	NS
TEST 2.								
Resting	13.1	23	2.1	10.4	3	2.1	2.106	<.025
Occluded	24.7	23	5.7	21.2	3	8.6	0.941	NS
Increment	11.6	23	4.5	10.8	3	6.8	0.268	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in 30-39 year old normal lipid, non-smoking patients and in 30-39 year old, normal lipid, non-smoking controls at Test 1 and at Test showing the t and P values for the comparison of patients and controls at each test point.

TABLE 115.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	11.4	13	2.1	10.3	24	3.0	1.200	NS
Occluded	23.2	13	6.5	21.4	21	7.9	0.693	NS
Increment	11.8	13	6.2	10.9	21	6.9	0.386	NS
TEST 2.								
Resting	11.1	9	2.0	10.6	11	1.3	0.708	NS
Occluded	24.0	10	4.7	22.7	10	7.0	0.484	NS
Increment	12.1	9	4.6	11.9	10	6.4	0.073	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in 40-49 year old normal lipid, non-smoking patients and in 40-49 year old, normal lipid, non-smoking controls at Test 1 and at Test showing the t and P values for the comparison of patients and controls at each test point.

TABLE 116.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.3	12	2.5	11.2	43	2.2	1.443	NS
Occluded	25.1	12	4.9	23.7	41	8.4	0.530	NS
Increment	12.8	12	4.6	12.6	41	7.2	0.118	NS
TEST 2.								
Resting	11.6	8	3.3	12.4	27	1.6	0.949	NS
Occluded	24.2	8	6.1	26.5	26	6.4	0.911	NS
Increment	12.5	8	3.4	14.1	26	5.9	0.723	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in 50-59 year old normal lipid, non-smoking patients and in 50-59 year old, normal lipid, non-smoking controls at Test 1 and at Test showing the t and P values for the comparison of patients and controls at each test point.

TABLE 117.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting.	12.4	33	3.1	10.6	44	3.1	2.51	<.01
Occluded	23.7	33	7.2	22.7	43	8.3	0.54	NS
Increment	11.1	33	5.2	12.1	43	7.2	0.51	NS
TEST 2.								
Resting	12.1	22	2.4	10.8	19	2.3	1.82	<.05
Occluded	23.3	22	7.0	25.0	17	7.1	0.75	NS
Increment	11.2	22	5.7	14.2	17	6.6	1.50	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in all normal lipid, smoking patients and in all normal lipid, smoking Controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 118.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.8	9	2.9	10.1	2	0.1	1.091	NS
Occluded	21.1	9	5.7	19.1	2	8.3	0.420	NS
Increment	8.6	9	4.5	9.0	2	8.2	0.104	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in 30-39 year old normal lipid smoking patients and in 30-39 year old normal lipid smoking controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 119.

F.A.	CONTROLS			PATIENTS				
Lysis-mm	$\bar{x}$	n	SD	$\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.1	16	3.2	10.3	22	3.5	1.662	NS
Occluded	23.5	16	7.9	22.1	22	8.9	0.494	NS
Increment	11.4	16	6.2	11.8	22	7.6	0.196	NS
TEST 2.								
Resting	11.0	8	1.0	11.6	7	3.2	0.500	NS
Occluded	22.1	8	7.2	23.0	6	8.6	0.217	NS
Increment	11.0	8	6.5	11.0	6	6.9	-	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in 40-49 year old normal lipid, smoking patients and in 40-49 year old normal lipid, smoking controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

TABLE 120.

F.A. Lysis-mm	CONTROLS $\bar{x}$	n	SD	PATIENTS $\bar{x}$	n	SD	t	P
TEST 1.								
Resting	12.8	8	3.3	11.0	20	2.9	1.406	NS
Occluded	26.1	8	7.3	23.8	19	8.1	0.688	NS
Increment	13.3	8	5.2	12.7	19	7.1	0.220	NS
TEST 2.								
Resting	14.0	6	2.3	10.3	12	1.5	4.132	<.0005
Occluded	26.6	6	7.0	26.1	11	6.3	0.149	NS
Increment	12.6	6	5.8	15.9	11	6.1	1.076	NS

Mean fibrinolytic activity (F.A. diameter of lysis -mm) resting, after 15 minutes venous occlusion, and the increment (occluded-resting) in 50-59 year old normal lipid, smoking patients and in 50-59 year old normal lipid, smoking controls at Test 1 and at Test 2 showing the t and P values for the comparison of patients and controls at each test point.

DISCUSSION.

As previously discussed in Chapter IV many workers have been interested in the possibility that a relationship exists between obstructive arterial disease and impaired fibrinolytic activity. To date, however, there is no conclusive evidence that such a relationship does exist. In the preceding chapters (VII and VIII) fibrinolytic activity, as demonstrated in euglobulin fractions applied to fibrin plates, has been shown to be related to a number of factors generally accepted as being C.H.D. "risk factors" - age, plasma lipid levels and blood pressure.

In this Chapter, evidence has been sought to confirm or refute the existence of a relationship between the level of observed fibrinolytic activity and the presence of C.H.D. by comparing groups of patients and controls matched for "risk factors".

Accepting that it is impossible to recruit exactly comparable patient and control groups, groups of healthy men apparently clinically free from C.H.D. were compared with men of similar age, smoking habit, blood pressure and plasma lipid status.

Generally, resting plasma fibrinolytic activity was found to be lower in the patients with C.H.D. than in the controls. Unfortunately, this did not always reach statistical significance (Table 121), although with two

exceptions, the finding of a lower mean resting plasma fibrinolytic activity in the C.H.D. patients than in the corresponding healthy controls was reproducible. The inability to gain statistical significance may have been due to insufficient numbers in the groups being compared.

This finding of lower resting plasma fibrinolytic activity in C.H.D. patients than in healthy men does not seem to be explained merely by the greater incidence of men with elevated lipid levels in the patient group than in the control group nor by the differing age distribution in the total groups, since even when aged-matched groups of patients and controls with normal plasma lipid levels are compared, the patients still have lower mean levels.

Plasma fibrinolytic activity after 15 minutes venous occlusion was usually lower in the patients than in the controls at Test 1 but this was statistically significant only when the total groups of patients and controls were compared and in the total groups of 30-39 year olds (Table 122).

This finding of lower post-occlusion fibrinolytic activity in the patients than in the controls could not be confirmed at Test 2. The reason for this is not clear. Fewer subjects were tested at Test 2 and although every effort was made to retest as many subjects as possible, only 75% of the controls and 51% of the patients tested at Test 1 were re-examined at Test 2.

In agreement with the observations of other workers (Chakrabarti et al 1968, Rosing et al 1973), we have found that mean resting plasma fibrinolytic activity is lower in

men with C.H.D. than in healthy men. Furthermore, we have demonstrated that this lower fibrinolytic activity seems to be independent of coexisting C.H.D. "risk factors". Unfortunately, the study model does not allow any comment on whether this reduced fibrinolytic activity is important in the aetiology of C.H.D.

The development and subsequent persistence of thrombi may be the result of imbalance between fibrin formation and dissolution. If this is so then individuals with defective fibrinolysis may be at increased risk of developing coronary atherosclerosis. Our observations would support this hypothesis. However, they do not exclude the possibility that the lower fibrinolytic activity observed in the patients may be the result of the disease process (rather than part of the cause). Widespread atherosclerosis may be associated with poor endothelial health and reduced activator production or release. Examination of this particular problem would require a large prospective study.

Rosing et al (1973) found that patients with C.H.D. had a reduced diurnal increase in fibrinolytic activity, but the difference which he demonstrated was not statistically significant. The increase in fibrinolytic activity in response to venous occlusion is normally very much greater than the increase due to the physiologic stimulus of diurnal rhythm. It was postulated that if patients with C.H.D. do indeed have defective fibrinolytic activity this may be detected by a reduced capacity in response to venous occlusion. Although it appears that

patients with C.H.D. respond less well to venous occlusion than healthy men do, we certainly have not demonstrated this convincingly.

A dilemma exists. C.H.D. is associated with reduced fibrinolytic activity, but, although it is an extremely attractive hypothesis, there is no proof that impaired fibrinolysis is important in the aetiology or pathogenesis of C.H.D. Therefore, although pharmacologic enhancement of fibrinolytic activity is possible, it would be inappropriate to suggest the widespread use of synthetic fibrinolytic agents. Clearly, however, attention must be paid to the correction of modifiable C.H.D. "risk factors" in the anticipation that amongst the benefits of lowering blood pressure, reducing body weight and normalising plasma lipid levels, there will be a physiological increase in fibrinolytic activity.

TABLE 121.

	All Ages P	30-39 Years P	40-49 Years P	50-59 Years P
Total Groups				
TEST 1	↓ <.005	↓ <.005	↓ <.01	↓ <.0025
TEST 2	↓ <.025	↓ NS	↓ NS	↓ NS
Normal Lipid Subjects				
TEST 1	↓ <.0005	↓ <.005	↓ <.025	↓ <.025
TEST 2	↓ <.05	↓ <.05	↓ NS	↓ NS
Normal Lipid Non-Smokers				
TEST 1	↓ <.0025	↓ <.01	↓ NS	↓ NS
TEST 2	↓ NS	↓ <.025	↓ NS	↑ NS
Normal Lipid Smokers				
TEST 1	↓ <.01	↓ NS	↓ NS	↓ NS
TEST 2	↓ <.05	-	↑ NS	↓ <.0005
Abnormal Lipid Subjects				
TEST 1	↓ <.05	-	↓ NS	↓ <.01
TEST 2	↓ NS	-	↓ NS	↓ NS

Resting plasma fibrinolytic activity healthy controls compared with C.H.D. patients at two test points and showing the P values of the results of comparison of total groups, subjects with normal lipids, normal lipid non-smokers, normal lipid smokers and abnormal lipid subjects.

↓ indicates C.H.D. patients had lower mean level than corresponding controls.

↑ indicates C.H.D. patients had higher mean level than corresponding controls.

TABLE 122.

	All Ages	P	30-39 Years	P	40-49 Years	P	50-59 Years	P
Total Groups								
TEST 1	↓	<.05	↓	<.025	↓	NS	↓	NS
TEST 2	↑	NS	↓	NS	↓	NS	↑	NS
Normal Lipid Subjects								
TEST 1	↓	NS	↓	NS	↓	NS	↓	NS
TEST 2	↑	NS	↓	NS	↓	NS	↑	NS
Normal Lipid Non-Smokers								
TEST 1	↓	NS	↑	NS	↓	NS	↓	NS
TEST 2	↑	NS	↓	NS	↓	NS	↑	NS
Normal Lipid Smokers								
TEST 1	↓	NS	↓	NS	↓	NS	↓	NS
TEST 2	↑	NS		-	↑	NS	↓	NS
Abnormal Lipid Subjects								
TEST 1	↓	NS	↓	NS	↓	NS	↓	NS
TEST 2	↓	NS		-	↑	NS		NS

Resting plasma fibrinolytic activity after 15 minutes venous occlusion, healthy controls compared with C.H.D. patients at two test points and showing the P values of the results of comparison of total groups, subjects with normal lipids, normal lipid non-smokers, normal lipid smokers and abnormal lipid subjects.

↓ indicates C.H.D. patients had lower mean level than corresponding controls.

↑ indicates C.H.D. patients had higher mean level than corresponding controls.

REFERENCES.

CHAKRABARTI, R., HOCKING, E.D., FEARNLEY, G.R., MANN, R.D., ATWELL, T.N., & JACKSON, D. (1968). Fibrinolytic activity and coronary heart disease. Lancet, 1, 987.

ROSING, D.R., REDWOOD, D.R., BRAKMAN, P., ASTRUP, T. & EPSTEIN, S.E. (1973). Impairment of the diurnal fibrinolytic response in man. Effects of ageing, Type IV hyperlipoproteinaemia and coronary artery disease. Circulation Research, 32, 752.